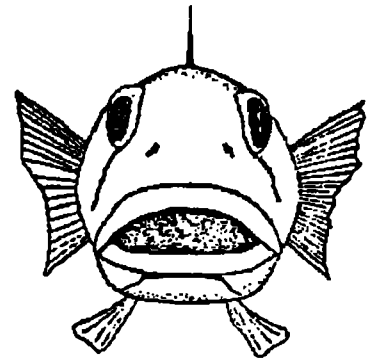


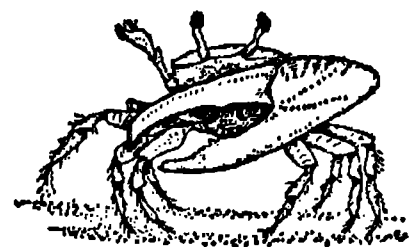
12th annual meeting



**Australian and New Zealand Society for
Comparative Physiology and Biochemistry**

University of Canterbury

**Christchurch, New Zealand
December 7-10 1995**



Organised by:

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PROGRAM

THURSDAY (7 Dec) EVENING

7.00 - 10.00pm

REGISTRATION AND INFORMAL SOCIAL, UNIVERSITY STAFF CLUB, ILAM ROAD.

FRIDAY (8 Dec) MORNING

8.30 **Registration, C Lecture Block Foyer**

9.00 **Introduction & Notices**

SESSION ONE, Lecture Theatre C2

Metabolic Depression and Torpor

9.10 **Metabolic depression in snails, frogs and bats: common patterns?**

P. Withers, Dept. of Zoology, University of Western Australia, Perth.

9.30 **The role of intrinsic factors, extrinsic effectors and energy-consuming processes in metabolic depression.**

M. Guppy, C.J. Fuery, J.E. Flanigan, S. Pedler & P.C. Withers, Depts. of Biochemistry and Zoology, University of Western Australian.

9.45 **Metabolic depression in Northern Territory burrowing frogs (*Cyclorana australis*).**

R. Godfrey, K. Mansfield, B.J. Wu & P. Else, Biomedical Science, University of Wollongong.

10.00 **Physiological variables and classification of torpor patterns in endotherms.**

F. Geiser, & T. Ruf, Dept. of Zoology, University of New England, Armidale; Dept. of Biological Sciences, Kent State University, Ohio, USA.

10.15 **Entry into daily torpor: time course of metabolic rate and body temperature reduction.**

X. Song, G. Körtner & F. Geiser, Dept. of Zoology, University of New England, Armidale, NSW.

10.30 **COFFEE & TEA BREAK**

SESSION TWO, Lecture Theatre C2

Circulation and Gas Exchange

11.00 **Keynote Address:**

Respiratory gas exchange in vertebrates: modeling based on structure and function.

J. Piiper, Max Planck Institute for Experimental Medicine, Göttingen, Germany.

11.40 **Molecular adaptations in fish hemoglobin function.**

R.E. Weber, Zoophysiology Department, University of Aarhus, Denmark.

12.10 **The significance of cardiac hypertrophy and other physiological changes in maturing male rainbow trout (*Oncorhynchus mykiss*).**

H. Thorarensen & P.S. Davie, Dept. of Physiology and Anatomy, Massey University, Palmerston North.

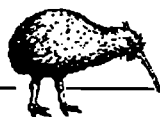
12.30 **Central cardiovascular shunts in the marsupial heart.**

S. Runciman, B.J. Gannon & R.V. Baudinette, Anatomy & Histology Dept. and School of Biological Science, Flinders University of South Australia, Adelaide.

12.45 **Seasonal variation in activity, body temperature, plasma proteins and haematology in free ranging active and hibernating Tasmanian echidnas, (*Tachyglossus aculeatus*).**

N.A. Andersen, D. Lovell, U. Mesch, K.M. Dziegielewska & S. Nicol, Dept. of Anatomy & Physiology, University of Tasmania, Hobart & Dept. of Hematology, royal Hobart Hospital.

1.00 **LUNCH - STAFF CLUB, ILAM HOMESTEAD, ILAM ROAD**



FRIDAY (8 Dec) AFTERNOON

SESSION THREE, Lecture Theatre C2

Body Temperature and Water Balance

- 2.15 Body temperatures of free-ranging wild dromedaries, *Camelus dromedarius*, in winter and summer, and deprived of water: a preliminary study.
B. Döriges, J. Heucke, G. Grigg & L. Beard, Dept. of Zoology, University of Braunschweig, Germany; Dept. of Zoology, The University of Queensland, Brisbane.
- 2.30 The circadian rhythm of body temperature of three marsupial species.
R. Gemmell, S. Turner & B. Krause, Dept. of Anatomical Sciences, The University of Queensland; Dept. of Pathology & Anatomical Sciences, The University of Missouri, USA.
- 2.45 The development of endothermy in the Tasmanian bettong (*Bettongia gaimardi*).
R. Rose, N. Kuswanti, S. Jones & E. Colquhoun, Depts. of Zoology & Biochemistry, University of Tasmania.
- 3.00 A comparison of oxygen consumption, ventilation and respiratory heat loss in two species of kangaroo, *Macropus rufus* and *Macropus giganteus*, in relation to ambient temperature.
C. Blaney, A. Krockenberger & T. Dawson, School of Biological Science, University of New South Wales.
- 3.15 Physiological adaptation to aridity in gerbils (Rodentia: Muridae: Gerbillinae).
P. Webb, Dept. of Zoology, University of Otago, Dunedin.
- 3.30 COFFEE & TEA BREAK

SESSION FOUR, Lecture Theatre C2

Physiology of Reproduction

- 4.00 Folliculogenesis in the Brushtail possum (*Trichosurus vulpecula*).
G.H. Shackell, N.G. Norman, B.J. McLeod & P.R. Hurst, AgResearch, Invermay Agricultural Research Centre, Mosgiel.
- 4.15 Do some species of *Niveoscincus* (Lacertilia: Scincidae) supplement autumn mating with a second mating in spring?
R. Swain, S.M. Jones & E. Wapstra, Dept. of Zoology, University of Tasmania, Hobart.
- 4.30 A novel animal model for investigation the aetiology of ovarian tumours?
B.J. McLeod, L.E. Fenton & T.R. Manley, AgResearch, Invermay Agricultural Research Centre, Mosgiel.
- 4.45 Plasma glucocorticoid concentrations in free-ranging platypus (*Ornithorhynchus anatinus*): seasonal patterns.
K.A. Handasyde, I.R. McDonald & B.K. Evans, Dept. of Zoology, University of Melbourne.
- 5.00 Plasma corticosterone levels are not significantly related to reproductive stage in female common geckos (*Hoplodactylus maculatus*).
J.E. Girling, & A. Cree, Dept. of Zoology, University of Otago, Dunedin.
- 5.15 Changes of sex steroid hormone profiles during artificial maturation of female New Zealand longfinned eels (*Anguilla dieffenbachii*).
P.M. Lokman & G. Young, Dept. of Zoology, University of Otago, Dunedin.
- 6.30 BUS LEAVES UNIVERSITY HALL, 9 MAIDSTONE ROAD FOR CONFERENCE DINNER (GONDOLA RESTAURANT).



SATURDAY (9 Dec) MORNING

SESSION FIVE, Lecture Theatre C2

Physiology of development

- 9.30 Ecophysiology of burrow-nesting in the Rainbow bee-eater (*Merops ornatus*).
A. Lill, Dept. of Ecology and Evolutionary Biology, Monash University.
- 9.45 Energetics of embryonic development in the Australian broad-shelled river turtle (*Chelodina expansa*).
D.T. Booth, Dept. of Zoology, The University of Queensland.
- 10.00 Lipid and protein metabolism in eggs of small skinks, *Morethia* and *Eumeces*.
M.B. Thompson, J.R. Stewart & K.J. Russell, School of Biological Sciences, University of Sydney & Faculty of Science, University of Tulsa.
- 10.15 The potential for matrotrophy in the viviparous skink, *Niveoscincus metallicus* from Tasmania.
S.M. Jones & R. Swain, Dept. of Zoology, University of Tasmania.
- 10.30 COFFEE & TEA BREAK

SESSION SIX, Lecture Theatre C2

Ionic Regulation & Transport

- 11.00 Sodium turnover in two land crabs, *Gecarcoidea natalis* and *Birgus latro* in rain forest on Christmas Island, Indian Ocean.
P. Greenaway, School of Biological Science, University of New South Wales.
- 11.15 Changes in the binding characteristics of natriuretic peptide receptors in the Atlantic hagfish (*Myxine glutinosa*) correlated with environmental salinity.
T. Toop, School of Biological and Chemical Sciences, Deakin University, Geelong.
- 11.30 Osmoregulation in the Port Jackson Shark, *Heterodontus portusjacksoni*, following hyposaline exposure.
A. Cooper & S. Morris, Biological Sciences, University of Sydney.
- 11.45 Intestinal Na⁺, K⁺-ATPase activity in chinook salmon: regional differences along the gut and the effect of seawater adaptation.
P.A. Veillette & G. Young, Dept. of Zoology, University of Otago, Dunedin.
- 12.00 A model for salt gland secretion in the green sea turtle, *Chelonia mydas*.
R. Reina, Division of Botany & Zoology, Australian National University, Canberra.
- 12.15 The effect of saline loading on renal function and plasma hormone levels in chickens.
A. Leary & J. Roberts, Dept. of Physiology, University of New England, Armidale, NSW.
- 12.30 The responses of the avian kidney to a reduction in renal mass.
I. Coulon & J. Roberts, Dept. of Physiology, University of New England, Armidale, NSW.
- 12.45 Effect of transport blockers on anion flux in the main duct of the parotid gland of red kangaroos.
A.M. Beal, School of Biological Science, University of New South Wales.
- 1.00 LUNCH, Students Association



SATURDAY (9 Dec) AFTERNOON

SESSION SEVEN, Lecture Theatre C2

Biochemistry & Molecular Biology

- 2.30 Fasting metabolism without ketosis: a novel role for glycerol in lactating Weddell seals.
G. Eisert, G.K. Barrell & M. Lever, Animal & Veterinary Sciences Group, Lincoln University; Christchurch & Canterbury Health Laboratories, Christchurch.
- 2.45 Energy Substrate Utilization by the Brush Tailed Possum (*Trichosorus vulpecula*).
M. Legge, B. McLeod, P. Mason, J. Crawford & G. Shackell, Dept. of Biochemistry, University of Otago, Dunedin; AgResearch, Invermay Agricultural Research Centre, Mosgiel.
- 3.00 The role of neopterin release by monocytes during inflammation.
S. Gieseg, G. Reibnegger, H. Wachter, & H. Esterbauer, Department of Zoology, University of Canterbury, Christchurch; Institute für Medizinische Chemie und Biochemie, Universität Innsbruck, Austria; Institute für Biochemie, Universität Graz, Austria.
- 3.15 Whey proteins genes of the common brush tailed possum.
C. Pottie, M. Ginger & M.R. Grigor, Dept. of Biochemistry, University of Otago, Dunedin.
- 3.30 Isolation and characterization of the vasopressin-like gene sequence in the lobster *Jasus edwardsii*.
J. Khoo & F.Y.T. Sin, Dept. of Zoology, University of Canterbury, Christchurch.
- 3.45 Hormonal Control of blood volume in Amphibia.
J.A. Donald, School of Biological and Chemical Sciences, Deakin University.
- 4.00 **POSTERS AND SOCIAL (Foyer and Mezzanine C Lecture Block; List of titles at the end)**

EVENING FREE

SUNDAY (10 Dec) MORNING

SESSION EIGHT, Lecture Theatre C2

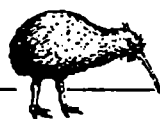
General Comparative Physiology

- 9.30 The evolution of sleep in mammals: the significance of the dreams of monotremes.
S. Nicol, N.A. Andersen & R. Berger, Dept. of Anatomy & Physiology, University of Tasmania; Dept. of Biology, University of California, Santa Cruz.
- 9.45 Mechanoreception in the snout of the echidna (*Tachyglossus aculeatus*).
E. Gregory, A. Iggo & U. Proske, Dept. of Physiology, Monash University.
- 10.00 Does Chromatic Aberration Limit Visual Resolution in the Fish?
W.S. Jagger, Dept. of Ecology and Evolutionary Biology, Monash University.
- 10.15 Intracellular freezing in *Panagrolaimus davidi*, an Antarctic nematode.
D.A. Wharton, D.J. Ferns, W. Block & H. Ramløv, Dept. of Zoology, University of Otago; British Antarctic Survey, Natural Environment Research Council, UK; Chemical Institute, University of Copenhagen, Denmark.
- 10.30 **STUDENT PRIZE AWARDS, VENUES FOR 1996, 1997, OTHER BUSINESS (if any).**
- 10.45 **COFFEE & TEA BREAK**



Muscle Physiology

- 11.15 Limiting factors for transient pH regulation in muscle tissues: perfusion and buffering characteristics.
N. Heisler, Dept. Animal Physiology, Humboldt Universität, Berlin.
- 11.45 Histidine dipeptides in monotreme muscle: physiological and phylogenetic implications.
J. Baldwin, Dept. of Ecology & Evolutionary Biology, Monash University.
- 12.00 *In Vivo* muscle force and elastic energy storage during load carrying in Tammar Wallabies.
R.V. Baudinette, A.A. Biewener, Biological Sciences, Flinders University, Adelaide; Organismal Biology and Anatomy, The University of Chicago, USA.
- 12.20 Implications of body size on the metabolic costs of locomotion in the Macropodoidea.
M.B. Bennett, Dept. of Anatomical Sciences, University of Queensland.
- 12.35 Muscle action during ballistic movement in an Antarctic fish, *Notothenia coriiceps*.
C.E. Franklin, & I.A. Johnston, Department of Zoology, University of Queensland; Gatty Marine Laboratory, University of St. Andrews, Scotland.
- 12.50 FAREWELL
- 1.00 LUNCH, Students Association



POSTERS

1. The effects of an omega-3 fatty acid supplement on growth and metabolism in juvenile tuatara (*Sphenodon punctatus*).

T. Blair, Dept. of Zoology, University of Otago, Dunedin.

2. Thermoregulation, thyroid function and catecholamines in the Brown Antechinus *Antechinus stuartii* (Marsupialia: Dasyuridae).

D. Schmidt & A. Bradley, Dept. of Anatomical Sciences, University of Queensland, Brisbane.

3. Reproduction in the Big-footed Bat *Myotis moluccarum* (Vespertilionidae).

S. Lloyd, L. Hall & A. Bradley, Dept. of Anatomical Sciences, University of Queensland, Brisbane.

4. The effect of hypothyroidism on olfactory function and the reproductive axis in adult male Wistar rats.

B. Ross & A. Bradley, Dept. of Anatomical Sciences, University of Queensland, Brisbane.

5. Internal volume and pressure regulation in the Paddle Crab, *Ovalipes catharus*.

S. Condliffe & H.H. Taylor, Department of Zoology, University of Canterbury, Christchurch.

6. The energetic cost of ventilation in the sand burrowing crab *Ovalipes catharus* (White, 1843) Brachyura: Portunidae.

G.W. Davidson & H.H. Taylor, Dept. of Physiology & Anatomy, Massey University, Palmerston North & Dept. of Zoology, University of Canterbury, Christchurch.

7. Muscle function in the hindlimb of kangaroos.

M. Raad & T. Dawson, School of Biological Science, University of New South Wales, Sydney.

8. Serotonergic neurons and the development of the antennal lobe of the brain of the honey bee (*Apis mellifera*).

D. Ferns, Dept. of Zoology, University of Otago, Dunedin.

9. Lactate dehydrogenase from Antarctic fish.

R. Fleming, D. Crossman & C. Marshall, Dept. of Biochemistry, University of Otago, Dunedin.

10. Measurements of oxygen partial pressures in the blood of an Antarctic fish, *Pagothenia borchgrevinki*.

M. Axelsson, W. Davison, M. Forster & S. Nilsson, Dept. of Zoology, University of Canterbury & Department of Zoophysiology, University of Göteborg, Sweden.

11. Casein genes of the common brush-tailed possum (*Trichosurus vulpecula*).

M. Ginger, C. Pottle & M. Grigor, Dept. of Biochemistry, University of Otago, Dunedin.

12. Uric acid metabolism in *Gecarcoidea natalis*.

S. Linton & P. Greenaway, School of Biological Science, University of New South Wales.

13. Aspects of the respiratory biology of two New Zealand intertidal fishes, *Acanthoclinus fuscus* and *Forsterygion* sp.

J.V. Hill, W. Davidson and I. Marsden, Department of Zoology, University of Canterbury, Christchurch.

14. Oxygen requirements of developing eggs of intertidal crabs.

N. Leelapiyanart & H.H. Taylor, Department of Zoology, University of Canterbury, Christchurch.

15. Are stress responses related to social rank in the grey duck (*Anas superciliosa*) and the laying hen (*Gallus domesticus*)?

K.E. Littin, R.M. Osborne, & J.F. Cockrem, Dept. of Physiology & Anatomy, Massey University, Palmerston North.

16. Seasonal changes in the renal morphology of *Antechinus stuartii* (Marsupialia: Dasyuridae).

B.M. McAllan, J.R. Roberts & T. O'Shea, Dept. of Physiology, University of New England, Armidale.



17. Natriuretic peptide receptors in the kidney of the toad, *Bufo marinus*.
S. Meier & J.A. Donald, School of Biological and Chemical Sciences, Deakin University.
18. Muscle degeneration in wild emus.
A. Patak & J. Baldwin, Dept. of applied Science, Edith Cowan University & Dept. of Ecology and Evolutionary Biology, Monash University.
19. The rhythm of life experienced by the New Zealand freshwater crayfish *Paranephrops zealandicus*
W.A. Titulaer & H.H. Taylor, Department of Zoology, University of Canterbury, Christchurch.
20. Ammonia/ammonium excretion and whole animal volume changes in the sea star (*Patiriella calcar*) following 24 h or hypo-osmotic and hypo-thermal stress.
G. Maas & T. Toop, School of Biological and Chemical Sciences, Deakin University.
21. The effects of captivity and of cortisol *in vitro* on estradiol-17 β production in greenbone (*Odax pullus*).
R.T. Wass & G. Young, Dept. of Zoology, University of Otago, Dunedin.
22. Effects of shore level and temperature on aquatic and aerial respiration of the mussel *Perna canaliculus*.
M.J. Weatherhead & I.D. Marsden, Department of Zoology, University of Canterbury, Christchurch.
23. Sodium transport mechanism in postmoult and Na-depleted crayfish *Cherax destructor*.
S. Zare & P. Greenaway, School of Biological Science, University of New South Wales.



Abstracts of Talks and Posters

In alphabetical order of presenting author

(underlined)

Seasonal variation in activity, body temperature, plasma proteins and haematology in free ranging active and hibernating Tasmanian echidnas, (*Tachyglossus aculeatus*)

Niels A. Andersen, David Lovell, Ute Mesch, Kate M. Dziegielewska and Stewart Nicol*
Dept. of Anatomy & Physiology, University of Tasmania, Hobart 7001, Tasmania, Australia.

*Dept of Hematology Royal Hobart Hospital, Hobart 7000, Tasmania, Australia.

Echidnas are widespread throughout Australia, and in the cooler parts of their habitat they hibernate during winter. During hibernation body temperature is reduced from the "normal" temperature of 32 °C to around 5 °C and heart rate may fall below one beat/min. The hibernation period in our study area is from about April to September and hibernation bouts last 3-4 weeks and are interrupted by brief arousals. Newly captured echidnas have, compared to other mammals, high hematocrit and hemoglobin values ($52 \pm 3\%$, 182 ± 12 g/L respectively ($\bar{x} \pm \text{SD}$.) N = 18) which decrease during captivity (A. Bollinger & Backhouse, T.C. (1960) *Proc. Zool. Soc. Lond.* 135, 91-97). The high hematocrit and low heart rate during hibernation raise the question of how the blood flow is maintained. In a preliminary study Nicol (in *Platypus and Echidnas*, eds. Augée, M.L., 140-144, Sydney, 1992) reported that while echidnas had a high blood viscosity in summer, this fell during hibernation. We therefore decided to study changes in hematology and viscosity in a population of free ranging echidnas which had been equipped with radio transmitters. Hibernating echidnas had an overall decrease in hematocrit and hemoglobin concentration ($46 \pm 7\%$, 170 ± 24 g/L respectively ($\bar{x} \pm \text{SD}$.) N = 6, $p < 0.05$) but viscosity measured in blood adjusted to a hematocrit of 45% showed an increase in hibernating and post hibernating echidnas compared to summer values. We found some sex differences in hematology, particularly in the post hibernating animals. Plasma protein concentration was decreased in the hibernating echidnas compared to active echidnas: this coincided with the disappearance of a protein band at approximately 140 kD as estimated from PAGE electrophoresis.

Histidine Dipeptides in Monotreme Muscle: Physiological and Phylogenetic Implications

John Baldwin

Department of Ecology and Evolutionary Biology, Monash University, Clayton, Vic 3168 Australia

The histidine dipeptides anserine, carnosine and ophidine (= balenine) make major contributions to short term intracellular pH buffering capacity in hypoxic vertebrate skeletal muscle. The distribution of these compounds has been documented for many families of fish, amphibians, reptiles, birds and placental and marsupial mammals. However, this information was not available for monotemes.

The concentrations ($\mu\text{mol g}^{-1}$ muscle) of histidine dipeptides determined by HPLC of acid extracts from triceps muscle of platypus and echidna respectively were: anserine, 5.2 and 8.2; carnosine, 0.5 and 0.6. Ophidine was not detected in either animal.

Both the diving platypus and burrowing echidna may encounter environmental and work induced muscle hypoxia. Metabolic adjustments associated with these behaviours include similar non-bicarbonate intracellular pH buffering capacities of about 40 slykes in the triceps muscle. A major fraction of this buffering capacity can now be attributed to anserine.

Phylogenetic implications of histidine dipeptide distribution among mammals are considered, but interpretation is hampered by the absence of data from many marsupial families.



***In Vivo* muscle force and elastic energy storage during load carrying in Tammar Wallabies.**

R.V. Baudinette and A.A. Biewener

Biological Sciences, Flinders University, Adelaide, SA 5001 Australia and Organismal Biology and Anatomy,
The University of Chicago, Chicago IL 60637, USA

To evaluate the role of elastic energy recovery in wallabies hopping with an advanced pouch young, *in vivo* measurements of muscle-tendon forces and rates of oxygen consumption were measured as the animals exercised on a motor-driven treadmill at speeds of 3.5 and 4.5 m/s. The joey was simulated by placing in the pouch a load equal to 13 per cent of the mother's weight. Buckle force transducers were attached to the tendons of the gastrocnemius, plantaris and flexor digitorum longus, the structures most important in the storage and subsequent recovery of elastic strain energy (Biewener and Baudinette: *J. expt. Biol* 198, 1829-1841). Muscle forces and elastic energy storage increased significantly between the unloaded and loaded condition at each of the two speeds. However rates of oxygen consumption were indistinguishable between either the loaded or unloaded conditions at either speed. The results indicate that load - (joey) carrying in these animals is essentially "free" and analogous to the characteristics of a simple spring. Our results will be discussed with reference to the belief that kangaroos and wallabies are the only known large hopping, bipedal animals.

Effect of transport blockers on anion flux in the main duct of the parotid gland of red kangaroos

A. Michel Beal,

School of Biological Science, University of New South Wales

When perfused with a solution having electrolyte concentrations similar to those of high-rate cholinergically-stimulated saliva (viz. Na, 159; K, 6; Cl, 6; HCO₃, 145; PO₄, 7 mmol/l plus a small amount of inulin), the main excretory duct of the resting parotid gland of Na-replete kangaroos reabsorbs HCO₃, PO₄ and K; secretes Cl, Ca and Mg; and transports negligible amounts of Na. As the fluxes of Cl and HCO₃ were approximately equal but opposite, and there was little concurrent change in total cation concentration or duct potential, direct coupling of Cl/HCO₃ transport was possible. The effects of transport blocking drugs and of anion substitutions in the perfusate on the fluxes of Cl and HCO₃ across the wall of the parotid main duct Na-replete kangaroos was investigated by *in situ* duct perfusion at 5.5 μl/min.

Incorporation of SITS ($n = 4$) into the duct perfusate at concentrations up to 1 mmol/l had no effect on the fluxes of Cl or HCO₃. Likewise, amiloride at 0.5 mmol/l ($n = 4$) or bumetanide at 0.5 mmol/l ($n = 4$) in the perfusate were without effect. Concentrations of methazolamide at 1 mmol/l perfusate ($n = 3$) were associated with small falls in Cl influx (11%; paired $t = 5.47$; $P < 0.05$) and HCO₃ efflux (11%).

Intracarotid infusion of SITS ($n = 3$) at rates sufficient to give an initial carotid plasma concentration of 0.5 mmol/l caused no significant changes in Cl and HCO₃ fluxes. Similarly, carotid plasma concentrations of methazolamide (> 0.75 mmol/l; $n = 3$), bumetanide (> 0.1 mmol/l; $n = 3$) or amiloride (> 0.25 mmol/l; $n = 3$) were without effect.

Total replacement of HCO₃ in the perfusate with gluconate ($n = 3$) did not alter Cl influx across the duct wall. However, HCO₃ flux was reversed and both Na concentration (paired $t = 6.05$; $P < 0.05$) and osmolality of the duct effluent were increased. Total replacement of perfusate HCO₃ with Cl ($n = 4$) caused reversal of direction of the Cl and HCO₃ fluxes.

These results provide no support for the involvement of the Cl/HCO₃ or Na/H antiports or the Na-K-2Cl symport in the movement of Cl and HCO₃ across the wall of the parotid main duct. Alternative explanations involve anion specific channels.



Implications of body size on the metabolic costs of locomotion in the Macropodoidea

M.B. Bennett

Department of Anatomical Sciences, University of Queensland, Australia

Medium to large macropods have been shown to be able to uncouple aerobic metabolic energy costs from the speed of locomotion. This makes hopping an economical gait, particularly at relatively high speeds. It is thought that this ability, which may be unique to macropod marsupials, is due to the utilisation of passive tissue elasticity to offset locomotor costs. During the first half of the ground contact phase, kinetic energy lost from the body is stored as elastic strain energy, predominantly in the hind limbs. The subsequent recoil returns kinetic and potential energy to the body.

I show that the allometry of structures in the legs and feet of macropods is different to the allometry of analogous features in quadrupedal eutherian mammals. The strong positive allometric scaling of tissue elastic energy storage potential in hoppers can partially explain the observed locomotor energetic results. However, a more complete explanation can be found if locomotor postures, locomotor performances, muscle-tendon moment arms about the ankle joint and body sizes are considered. My findings demonstrate how the utilisation of tissue elasticity is strongly mass-dependent and help explain why 'large' macropods can uncouple oxygen consumption from the speed of locomotion, whereas small macropods cannot.

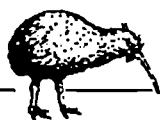
The results of this study also provide a possible reason for the 'upper limit' of body mass found in extant macropods, and may help us interpret the locomotor performance of extinct giant kangaroos and wallabies.

The effects of an omega-3 fatty acid supplement on growth and metabolism in juvenile tuatara (*Sphenodon punctatus*).

Tracy Blair

Department of Zoology, University of Otago

Tuatara (*Sphenodon*) are the sole surviving member of the Order Sphenodontida and are endemic to New Zealand. They are now restricted to about 30 offshore islands. A national recovery plan was established in 1993. One of the major goals was to establish a self-maintaining captive population of tuatara. However, high juvenile mortality, obesity and abnormal growth are all common occurrences and hence this goal is yet to be achieved. Diet and nutrition of captive tuatara have been implicated as the major causes of these occurrences. Differences are known to occur between diets of wild and captive tuatara. Seabirds provide the only source of long chain fatty acids (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) to wild tuatara, however, captive tuatara do not receive seabirds nor any prey item containing these fatty acids. The importance of nutrients received from seabirds was recognised as an area needing investigation. This experiment tests the hypothesis that supplementation with an omega-3 fatty acid will effect growth rate, metabolism, plasma concentration of total cholesterol and triacylglycerol and fatty acid composition of phospholipids in red blood cells in captive tuatara. Eighteen juvenile tuatara will be housed singly and fed insect diets. Nine will receive an omega-3 fatty acid supplement (containing EPA and DHA) in the form of fish oil whereas the remaining nine will receive an olive oil supplement. Certain aspects of this experiment will be discussed.



A comparison of oxygen consumption, ventilation and respiratory heat loss in two species of kangaroo, *Macropus rufus* and *Macropus giganteus*, in relation to ambient temperature.

Cindy Blaney, Andrew Krockenberger and Terence Dawson
School of Biological Science, University of New South Wales

A preliminary study assessed oxygen consumption, ventilation and respiratory heat loss in two species of kangaroo, *Macropus rufus* and *M. giganteus*, in relation to ambient temperature. The two species of kangaroo provided a comparison between an inhabitant of arid and semi-arid environments, *M. rufus*, and an inhabitant of predominantly mesic environments, *M. giganteus*.

At the cooler ambient temperatures, -5°C , 5°C and 15°C , there were no significant differences between the two species of kangaroo. In the thermoneutral zone (25°C) *M. giganteus* had a significantly higher minute volume (V_i) than *M. rufus*. *M. rufus* had a significantly greater respiratory frequency (f_R) at 33°C but not at 45°C . It is interesting to note that despite being an inhabitant of more arid habitats *M. rufus* had significantly greater evaporative water loss at 45°C . However, neither species were water restricted. The results indicate that *M. rufus* begins to dump heat at lower ambient temperature than *M. giganteus*.

Energetics of embryonic development in the Australian broad-shelled river turtle (*Chelodina expansa*).

David T. Booth

Department of Zoology, The University of Queensland

The broad-shelled river turtle has an exceptionally long natural incubation period for a Chelonian (over 320 days). My investigations indicate that three factors appear to contribute to this prolonged incubation: 1. low incubation temperature, 2. incubation diapause, and 3. an inherently slow rate of embryonic development. I studied the energetics of embryonic development by monitoring oxygen consumption of growing embryos throughout incubation at 26°C and 28°C . Overall patterns of oxygen consumption were similar at both temperatures, but eggs incubated at 26°C took an average of 173 days to hatch, while those incubated at 28°C only 142 days. However, the "growth phase" of development appeared to be the same at both temperatures, the difference in incubation period being due to difference in the diapause period. The production efficiency of embryonic development in terms of mL of oxygen consumed per gram of yolk-free hatchling tissue produced was $114 \text{ mL O}_2/\text{g}$ at both incubation temperatures.



Reproduction in the Big-footed Bat *Myotis moluccarum* (Vespertilionidae).

Shan Lloyd & Les Hall & Adrian Bradley

Department of Anatomical Sciences, University of Queensland, Brisbane

In response to adverse weather conditions many microchiropterans living in cool temperate climates frequently enter hibernation, decreasing nutritional requirements and thus metabolism. However, the reduction in core body temperature also affects biochemical processes and therefore endocrine events and embryonic growth are considerably slowed. A number of reproductive strategies are found in the family Vespertilionidae which ensure that reproduction is successful. While these strategies are most fully developed in cool temperate dwelling species they are, in a reduced form, evident in tropical and subtropical species also. The aim of this research was to determine if an 'intermediate' pattern of vespertilionid reproduction was responsible for some unusual observations occurring in the warm temperate polyoestrous Vespertilionid *Myotis moluccarum* in SE Qld. Levels of testosterone in males and progesterone in females were determined by radioimmunoassay on 40µl blood samples taken each month from ten females and five males. Approximately two females were sacrificed each month to determine stage of pregnancy and/or follicular growth as well as to detect the presence and possible storage of spermatozoa in the female reproductive tract over winter. Testosterone concentrations remained high from July to November with a significant decline occurring in December. Females underwent ovulation in early August but the presence of sperm in the reproductive tract of a July specimen indicates that mating may commence prior to ovulation. The second pregnancy commenced soon after parturition in late October to early November. The typical vespertilionid follicle of hibernation was apparent but for only a brief period prior to ovulation. Progesterone levels increased throughout pregnancy from a minimum of < 1 ng/ml before pregnancy to a maximum of 95.6 ng/ml in late pregnancy.

The effect of hypothyroidism on olfactory function and the reproductive axis in adult male Wistar rats.

Brett Ross & Adrian Bradley

Department of Anatomical Sciences, University of Queensland, Brisbane

In several studies a clear relationship has been shown to exist between olfactory function and the activity of the pituitary-thyroid axis. In man most of these interactions of thyroid status and olfactory function have been made in patients exhibiting hypothyroidism. In animal studies propylthiouracil induced hypothyroidism has been shown to induce anosmia. To further investigate such findings in this study on Wistar rats methimazole, a known goitrogen, was used to induce a state of hypothyroidism. Methimazole was administered to 14 rats in drinking water (25mg/500ml) for 57 days. The dose was then doubled from day 57 to day 84. Food seeking ability was used to assess olfactory function at a behavioural level while the expression of the *Fos* protein in the nuclei of the granular cell layer of the olfactory bulb following propionic acid stimulation was used to assess the level at which thyroid hormone deprivation may act on olfactory function. Measurement of plasma total T4 concentrations showed a significant decline from 7.6 µg/dl in the control group to 4.9 µg/dl at day 50, 2.3 µg/dl at day 69 and 2.9 µg/dl at day 84. No behavioural difference was observed since the ability to locate a buried food pellet remained unaffected. At the level of the olfactory bulb no difference was observed in the morphological expression of *Fos* following stimulation between hypothyroid and euthyroid animals. There was, however, a 12% decrease in olfactory epithelial thickness in hypothyroid animals. Basal plasma testosterone concentrations were unaffected by thyroid status however capacity to respond to a 10µg GnRH challenge was significantly elevated. This was correlated with significant increases in the size of both Leydig cells and Sertoli cells nuclei.



**Thermoregulation, thyroid function and catecholamines in the
Brown Antechinus *Antechinus stuartii* (Marsupialia:Dasyuridae).**

Danny Schmidt & Adrian Bradley

Department of Anatomical Sciences, University of Queensland, Brisbane

While marsupials do not have brown adipose tissue their thermoregulatory ability matches that of eutherian mammals. Previous studies have suggested that thyroid function is an important factor in cold acclimation in marsupials.

A study was carried out to investigate the relationship between thyroid function and thermoregulation with alterations in ambient temperature in the Brown Antechinus *Antechinus stuartii*. Wild caught Antechinus were administered 0.05% (w/v) methimazole in drinking water for a period of 11 weeks and held at a range of temperatures from 23 to 10°C. Methimazole dosed animals had significantly lowered plasma thyroxine (T₄) concentrations. The core body temperature of methimazole treated animals was consistently lower than that of the controls over the entire ambient temperature range. Thyroxine utilization rate (µg/day) was significantly reduced in the methimazole treated animals compared with the controls at all temperatures however there was no increase in either total T₄ or T₄ utilization rate as temperatures were decreased.

Plasma noradrenaline (NA) to adrenaline (A) ratio was always greater than 1, with NA ranging from 5 to 84 ng/ml and A ranging from 0.81 to 22 ng/ml. Compared with the controls there was a significant increase in the NA concentration in the methimazole treated animals.

These findings are consistent with NA serving a thermogenic role in hypothyroid marsupials.

Internal volume and pressure regulation in the Paddle Crab *Ovalipes catharus*

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The rigid exoskeletons enclosing crabs imply that relatively small changes in internal volume (eg haemorrhage) could cause drastic drop in internal pressure and/or distortion of compliant structures such as the gills. A pressor role has been proposed for the dorsoventral muscles (DVM) which extend between the carapace and the roof of the branchial chamber (Taylor et al 1992). Removal of a standard volume of haemolymph (3 mL/100g, about 12% haemolymph volume) from the pericardium caused an immediate (> 30s) increase in tonic DVM electromyogram (EMG) frequency from 3.8 ± 0.59 Hz to 8.67 ± 1.02 Hz. DVM frequency then decreased towards baseline, about 40% of the recovery occurring within 30 minutes. Drinking, inferred from mass changes, occurred at a high rate following haemolymph removal, compensating for the majority of the volume removed within 30 minutes. As mass was increasing, the extracellular volume (⁵¹Cr-EDTA space) also increased with a slower time course, suggesting ingested water was being transferred to the haemolymph. This was also supported by a decrease in the haemocyanin concentration consistent with dilution of the haemolymph. Interestingly, haemocyanin concentration was restored to its initial value by 24 hours suggesting mobilisation of an internal reserve. It is postulated that the DVMs mediate a short term baroreflex that serves to offset a sudden decrease in pressure following haemolymph removal. Decreases in haemolymph volume are restored over a longer term by transferring ingested water into the haemolymph.

Taylor, H.H., Davidson, G.W., Field, L.H. & Taylor, E.W. (1992) The dorsoventral muscles of crabs: controllers of hydrostatic pressure and gill blood flow? In: Hill, R.B, Kuwasawa, K & McMahon, B.R. Molecular and Comparative Physiology Vol. 11, Basel:Karger, pp37-50.



Osmoregulation in the Port Jackson Shark; *Heterodontus portusjacksoni*, following hyposaline exposure

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Juvenile *Heterodontus portusjacksoni* residing in shallow bays and estuaries are subject to marked fluctuations in seawater salinity. Following exposure to hyposaline water for 24h, plasma osmotic pressures were 980, 780, and 590 mmol. L⁻¹ in control (1000 mOsm. kg⁻¹ [100% SW]), 750 mOsm. kg⁻¹ [75% SW] and 500 mOsm. kg⁻¹ [50% SW] respectively. Plasma concentrations remained slightly hyperosmotic to the external environment for a further 168h. The main plasma osmolyte urea was reduced from 410 to 200 mmol. L⁻¹ after 168h in 50% SW. The other major organic osmolyte TMAO was also markedly reduced from 70 to 12 mmol. L⁻¹. Erythrocyte urea concentrations were similarly reduced from 380 to 195 mmol. L⁻¹ and erythrocyte TMAO from 92 to 24 mmol. L⁻¹. Both plasma [Na] and [Cl] were also markedly reduced from control concentrations of 290 to 195 mmol. L⁻¹ and from 289 to 180 mmol. L⁻¹ respectively, thus remaining hypo-ionic to the surrounding seawater. An increase in body weight after 6h exposure in 75% SW (8% increase) and 50% SW (15% increase) was partially compensated within 168h. Similarly, blood haematocrit and [Hb] were also markedly reduced within 6h before returning to pre-treatment values within 168h. The osmoregulatory strategy of *H. portusjacksoni* to hyposaline exposure thus appears to require rapid osmolyte reduction, mainly in the form of urea. The marked loss of plasma [Na] and [Cl] against the seawater/blood gradient while still maintaining relatively high urea concentrations suggest that this organic osmolyte plays an important part in cell homeostasis. The possible effects of acute reductions in plasma/seawater gradients on acid-base status will also be discussed.

The energetic cost of ventilation in a sand-burrowing crab, *Ovalipes catharus*

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O. catharus, like other crabs which burrow into soft sediments, exhibits prolonged periods of reverse ventilation. Following 15 minutes of swimming, crabs spent 67% of the time reverse-ventilating when buried in sand and 25% when unburied. Settled crabs reverse-ventilated continuously in both states.

The adaptive significance of reverse ventilation does not appear to be related to oxygen extraction efficiency ($E_w\%$) which was similar during adjacent periods of forward and reverse ventilation in buried crabs and higher for forward ventilation in unburied crabs. In unburied crabs, branchial chamber pressures (P_{branch}) were of similar magnitude in the two ventilatory modes (forward, -0.16 kPa; reverse, +0.14 kPa, at $V_w = 0.54$ and 0.45 L kg⁻¹ min⁻¹ respectively). P_{branch} was higher in buried crabs and highest in buried forwardly ventilating crabs (forward, -0.71 kPa; reverse, +0.26 kPa at 0.28 and 0.27 L kg⁻¹ min⁻¹ respectively). These differences reflect a higher resistance to ventilatory water flow in buried crabs, caused partly by the sediment (especially in forward ventilation), and partly by adjustment of the ventilatory apertures at the leg-bases.

The higher values of P_{branch} , increased the ventilatory power requirement when buried, especially when forwardly ventilating. Using experimentally determined relationships among V_w , P_{branch} and $E_w\%$, and values of scaphognathite muscle efficiency from the literature, ventilatory power as a fraction of metabolism was calculated for the four ventilation states. At $\dot{M}O_2$ of 50.5 $\mu\text{mol kg}^{-1} \text{min}^{-1}$, the ventilatory fractions were: unburied forward 8.7%; unburied reverse 26.5%; buried forward 64.6%; buried reverse 26.9%. It suggested that the increased dependence on reversed ventilation shown by burrowing crab species is related to lowering the overall cost of ventilation by reducing utilisation of the energetically expensive forward mode.



Muscle Function in the Hindlimb of Kangaroos

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We have examined the structure-function relationships of the muscles of kangaroos which are involved in hopping. Of note is the large m. caudofemoralis (423g/side) which is uniquely developed in the kangaroo; it does not occur in other large quadrupeds. With its acute angle of insertion and large lever arm, the m. caudofemoralis is adapted for fast and powerful movements. The function is intermediate between the other extensors of the thigh, the gluteal group (adapted for rapid movement) and the ischio-pubic complex (powerful but slower movement).

The muscles of the lower back are also unusually developed. The erector spinae form a large muscle group which aids in raising the anterior part of the body during take-off. The origin of this muscle group from the tuber coxae is different to the situation seen in other cursorial mammals and allows room for the uniquely developed multifidus lumborum. This muscle forms a large mass (349g/side) in the pelvis and it controls the counterbalancing of the tail during hopping.

In kangaroos the roles of the adductors and abductors are changed so that emphasis is placed on the muscles acting in a parasagittal plane. This arrangement allows more power to be provided in extending the thigh and propelling the body forward and up. The extended length of the ischium aids this action since the adductors (and hamstrings) are able to originate further back from the femur and have a larger area of origin.

Fibre typing of these muscles was undertaken to further our understanding of their functional characteristics. In the power muscles the proportion of type I fibres (slow oxidative) is low, 1-20%. There appears to be only one form of type II fibres, which are moderately oxidative. Such a pattern is seen also in dogs. This points to a generally high aerobic capacity.

Hormonal Control of Blood Volume in Amphibia

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Our knowledge of the endocrine control of water balance in amphibians is skewed towards the mechanisms that prevent dehydration in adult animals. Most adult amphibians spend time in fresh water in which they are confronted with an osmotic gain of water which, if uncompensated, will result in the expansion and dilution of the extracellular and intravascular fluid spaces. To date, there has not been any consideration of the hormonal mechanisms controlling volume load in amphibia. The natriuretic peptides (NPs) are a family of peptides that have been identified in species from each vertebrate class. In mammals, a number of studies have shown that NPs act to reduce intravascular volume loading by mediating renal natriuresis and diuresis, and hypotension. These effects are mediated by NP receptors (NPR) signalling via cGMP and possibly other signal transduction pathways. The aim of our research is to investigate the hypothesis that the NP endocrine system regulates blood volume in the cane toad, *Bufo marinus*. The response of the NP system to blood volume changes may be reflected by alterations in parameters such as NP gene transcription, the circulating level of the peptide, and the expression and kinetics of NPR. Currently, a range of molecular techniques are being used to clone the NP and NPR genes from various tissues of *B. marinus*. Partial clones of two types of NPR have been obtained and one was shown to have 80% homology with the mammalian NPR-C. The sequence of the second receptor is currently being obtained. cDNA library screening is being used to obtain the *Bufo* NP cDNAs using ANP cDNAs from the frog, *Rana ridibunda*, as a probe. Gene cloning will provide homologous cDNA probes for studies which will determine the relationship between blood volume and the expression of NP and NPR genes. In addition, the amino acid sequence of *Bufo* NPs will be deduced from the cDNA sequence, and peptides and antibodies synthesised for the development of a homologous radioimmunoassay system for the quantification of plasma and tissue peptide levels.



Fasting metabolism without ketosis: a novel role for glycerol in lactating Weddell seals

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During the lactation period, Weddell seals (*Leptonychotes weddelli*) maintain low plasma levels of ketone bodies ($<0.2 \text{ mmol.L}^{-1}$) in spite of substantial mobilisation of adipose tissue. To investigate the absence of fasting ketonaemia in these animals, carnitine, acetylcarnitine, glycerol and free fatty acids were measured in plasma samples from non-lactating ($n=9$) and lactating ($n=15$) seals, as well as their pups ($n=10$). Plasma carnitine levels ($9.76 \pm 0.56 \text{ } \mu\text{mol.L}^{-1}$, $n=34$) were low by human standards (normal range $30\text{-}40 \text{ } \mu\text{mol.L}^{-1}$) and there were no significant differences between groups. Plasma acetylcarnitine concentration was low also but was significantly higher in lactating animals ($3.10 \pm 0.56 \text{ } \mu\text{mol.L}^{-1}$, $P < 0.05$) than non-lactating females ($1.71 \pm 0.32 \text{ } \mu\text{mol.L}^{-1}$) and pups ($1.36 \pm 0.36 \text{ } \mu\text{mol.L}^{-1}$). Plasma glycerol concentration was higher ($P < 0.05$) in lactating ($355.36 \pm 26.01 \text{ } \mu\text{mol.L}^{-1}$) versus non-lactating ($231.89 \pm 25.25 \text{ } \mu\text{mol.L}^{-1}$) females, but lower than in pups ($621.33 \pm 98.04 \text{ } \mu\text{mol.L}^{-1}$). Free fatty acids were highest in lactating mothers ($1296 \pm 130 \text{ } \mu\text{mol.L}^{-1}$), followed by pups ($751 \pm 35 \text{ } \mu\text{mol.L}^{-1}$) and non-lactating females ($500 \pm 58 \text{ } \mu\text{mol.L}^{-1}$). The low plasma carnitine concentrations are consistent with prolonged fasting in the adults and the acetylcarnitine data indicate no accumulation of acetyl-CoA in mitochondria of these animals, making them unable to generate ketone bodies. Such absence of ketone body production means that glucose pools have to be supported at or close to non-fasting levels by gluconeogenesis during the lactation period. The plasma glycerol levels recorded here, together with the fatty acids data, are consistent with glycerol being the primary substrate for gluconeogenesis and it is proposed that under conditions of negative energy balance Weddell seals utilise glycerol for this purpose.

Serotonergic neurons and the development of the antennal lobe of the brain of the honey bee (*Apis mellifera*)

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The biogenic amine serotonin is a modulator of neuronal activity and behaviour in the adult central nervous system of vertebrates and invertebrates. Evidence suggests that serotonin may also have an important role as a regulator of neuronal development. The antennal lobes of the honey bee brain undergo massive structural change during metamorphosis, providing a model system for the study of neuronal development. In the adult, the antennal lobe consists of discrete, identifiable subunits, known as glomeruli. Most, if not all glomeruli in the antennal lobe are innervated by processes of a single large serotonin-immunoreactive (SIR) deutocerebral neuron. It is shown here that the SIR neuron is already present in the deutocerebrum at the beginning of metamorphosis and that the neuron has invaded the neuropil of the antennal lobe by the time the glomeruli are forming. From pupal stage 6 and beyond, processes of the SIR neuron can be distinguished within individual, identifiable glomeruli. This study shows that the serotonergic neuron is well placed to influence the development of the antennal lobes of the brain.



LACTATE DEHYDROGENASE FROM ANTARCTIC FISH

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Antarctic fish live constantly at subzero temperatures, but the enzymes in these fish still maintain an efficient rate of catalysis. These temperatures slow down biochemical activity such that the conformational movements associated with the binding of cofactors and substrates is unable to occur. This causes enzymes from temperate species to be inactivated at low temperatures but enzymes from Antarctic fish have adapted to these temperatures and are able to carry out efficient catalysis. Lactate dehydrogenase (LDH, EC 1.1.1.27) is a well studied enzyme and easily purified making it ideal for comparative studies. LDH has been extracted from white muscle of the Antarctic Notothenioid *Dissostichus mawsoni* and its more temperate relative *Paranotothenia angustata*. LDH has a number of isoenzymes which are found in different tissues and only one isoenzyme was isolated from the white muscle. The apparent K_m of the LDH from the two fish at different temperatures indicate that the K_m of the *D. mawsoni* is consistently higher than that of the *P. angustata*. Future work will include crystallizing the LDH and analyzing the resulting structure by X-ray crystallography, which will provide valuable information as to the structural differences associated with cold adaptation of enzymes. The DNA sequence will be obtained so that comparisons can be made with other organisms.

Measurements of oxygen partial pressures in the blood of an Antarctic fish, *Pagothenia borchgrevinki*.

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Department of Zoology, University of Canterbury and Department of Zoophysiology, University of Göteborg¹.

Under anaesthesia, afferent and efferent branchial arteries were cannulated and flow probes were placed around the ventral aortas of *Pagothenia borchgrevinki*. The fish were allowed 24 hours to recover from surgery. Swimming trials were performed on fish transported from McMurdo Sound to Christchurch and held at 0°C. Other experiments were performed at Scott Base. Using a Minipuls 3 peristaltic pump, blood was pumped from one branchial artery to another in a continuous extracorporeal loop (180 μ l) passing a Microelectrodes oxygen electrode. Flow could be reversed, allowing measurement of both PaO_2 and PvO_2 . Intraarterial injection of adrenaline produced an increase in both PaO_2 and PvO_2 . PvO_2 decreased on exercise. In half the animals PaO_2 increased on swimming and in half it fell. There was a strong negative correlation between PaO_2 and gill vascular resistance.



Muscle action during ballistic movements in an Antarctic fish, *Notothenia coriiceps*

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The power output of isolated muscle fibres operating under the actual constraints that occur *in vivo* in fish performing ballistic movements has been determined. High speed cinématography was used to investigate the kinematics of burst swimming (escape responses) in benthic Antarctic fish, *Notothenia coriiceps*. Whilst filming, synchronous sonomicrometry and electromyography recordings were made from myotomal muscle at rostral and caudal positions, providing *in vivo* measurements of muscle strain and activation (EMGs; phase and duty cycle). These *in vivo* muscle parameters were "played-back" to an isolated fibre preparation and power outputs determined using the work-loop technique.

Physiological variables and classification of torpor patterns in endotherms

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Hibernation and daily torpor are usually considered to be two distinct patterns of heterothermia. In the present comparison we evaluated (i) whether physiological variables of torpor from 104 avian and mammalian species warrant the distinction between two different states of torpor and (ii), if so, whether this distinction is best based on maximum torpor bout duration, minimum body temperature (T_b), minimum metabolic rate during torpor, or the reduction of metabolic rate expressed as % of basal metabolism (BMR). Initially, animals were grouped into species displaying either daily torpor or prolonged torpor (hibernation) using observations from the original sources, which was verified by both cluster and discriminant analyses.

All variables tested differed significantly ($p < 0.001$) between daily torpor and hibernation. The average maximum torpor bout duration was 355.3 h in hibernators and 11.2 h in daily heterotherms. Mean minimum T_b s were lower in hibernators than in daily heterotherms (5.8 °C vs 17.4 °C), as were minimum metabolic rates, measured as rate of oxygen consumption ($\dot{V}O_2$, 0.037 vs 0.535 mL O₂ g⁻¹h⁻¹), and the metabolic rate reduction expressed as % of BMR (5.1% vs. 29.5%). Furthermore, mean body weights were significantly higher in hibernators (2384 g) than in daily heterotherms (253 g). Thus, the comparisons of several physiological variables appear to justify a distinction between the two torpor patterns. However, of all variables tested, only the frequency distributions of maximum torpor bout duration (1.5-22 h daily torpor; 96-1080 h hibernation) showed a clear gap between daily heterotherms and hibernators. The minimum $\dot{V}O_2$ also distinguished clearly between daily heterotherms and hibernators. All other variables showed considerable overlap between the two groups. We therefore suggest that classification of torpor patterns should be based on maximum duration of torpor bouts or the minimum $\dot{V}O_2$ during torpor.



The circadian rhythm of body temperature of three marsupial species.

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Rectal thermocouples have been used to obtain body temperature measurements from restrained animals usually during the daylight hours. These single values represented the mean of a period of rest or were simply a stable or plateau value obtained during the day or night. With the advent of small temperature transmitters that can be placed in the body cavity of an animal, continuous monitoring of the body temperature of free-ranging animals can be achieved. The results of these studies have shown that the body temperature of many mammals varies throughout the day and night in a sinusoidal manner. In the present study, the 24 hour pattern of body temperature of the brushtail possum *Tirchosurus vulpecula*, the northern brown bandicoot *Isodon macrourus* and the Virginia opossum *Didelphis virginiana* was obtained using temperature transmitters (Titley Electronics, Ballina, NSW). Although all three species exhibited a circadian rhythm with the peak at midnight and a nadir at noon, the profile of the opossum was not as defined as those of the bandicoot and possum, with one opossum not showing a definite 24 hour rhythm. The mean body temperature of the possum, bandicoot and opossum was 37.4°C, 36.3°C and 35.4°C, respectively. It is of interest to note the variation in mean body temperatures between these three marsupial species, this variation in body temperature having also been reported in eutherian species. The reason for and the evolutionary advantage of having a certain body temperature is still a current area of discussion.

The role of neopterin release by monocytes during inflammation

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Neopterin and its reduced form, 7,8 dihydroneopterin are pteridines released from macrophages and monocytes when stimulated with γ -interferon *in vivo*. The function of this response is unknown though there is an enormous amount of information available on the use of these compounds as clinical markers of monocyte/macrophage activation. We have found that *in vitro*, 7,8-dihydroneopterin dramatically increases, in a dose dependent manner, the lag time of low density lipoprotein oxidation mediated by Cu^{++} ions or the peroxy radical generator 2,2'-azobis (2-amidino propane) dihydrochloride (AAPH). 7,8-Dihydroneopterin also inhibits AAPH mediated oxidation of linoleate. No interaction was found between 7,8NP and α -tocopherol during oxidation. The kinetic of the inhibition suggests that 7,8-dihydroneopterin is a potent chain breaking antioxidant which functions by scavenging lipid peroxy radicals.

These results strongly suggest that the purpose of 7,8NP synthesis and release by macrophages is to increase the intracellular and extracellular anti-oxidant protect of the cell membranes during inflammation.



Casein genes of the common brush-tailed possum (*Trichosurus vulpecula*)
Melanie Ginger, Christine Piotte and Murray Grigor
Department of Biochemistry, University of Otago

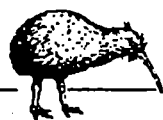
The casein fraction was isolated from possum milk by centrifugation and analysed by two dimensional polyacrylamide gel electrophoresis (2D-PAGE). When visualised in this manner, the casein fraction appears as a complex of spots representing proteins of between 36 and 39 kDa in molecular weight. The major protein components were identified by N-terminal amino acid sequence and by their homology to the known amino acid sequence of caseins of the tammar wallaby. N-terminal sequence analysis revealed that all of the spots observed were attributable to either α - or β -casein. This is consistent with post-translational modification of two main polypeptides. Both caseins are phosphorylated, however only α -casein is glycosylated. No changes were detected in either the electrophoretic pattern, or the level of expression of particular species as lactation progressed. Full-length cDNA clones of α - and β -casein have been isolated from a possum mammary gland cDNA library following screening with cDNA clones of milk protein genes of the tammar wallaby. These possum clones have had their full sequence determined and will be used as probes in northern analysis to characterise the expression of mRNA transcripts of these species throughout lactation.

**Plasma corticosterone levels are not significantly related
to reproductive stage in female common geckos
(*Hoplodactylus maculatus*)**

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We investigated the relationship between plasma concentrations of the adrenal "stress" steroid corticosterone and reproductive state in New Zealand's common gecko, *Hoplodactylus maculatus*. We hypothesised that seasonal variation in plasma corticosterone concentration might contribute to the prolonged gestation period (c. 14 mo) of a population from a cool region in which reproduction occurs biennially. Unexpectedly, concentrations did not vary significantly with season or reproductive stage. We also investigated the effects of time of day and capture stress on concentrations of plasma corticosterone. Concentrations were consistently low in both day and night samples and did not rise significantly when females were held up to 2.5 h in cloth bags. We conclude that plasma corticosterone concentration shows less variation in *H. maculatus* than in other reptiles studied and that there is no evidence of a role for corticosterone in maintaining the long gestation period in this biennially reproducing species.



Metabolic Depression in Northern Territory Burrowing Frogs (*Cyclorana australis*)

Robin Godfrey, Kylie Mansfield, Ben Jing Wu and Paul Else

The Australian burrowing frog (*Cyclorana australis*) is one of a number of ectothermic vertebrates that show metabolic depression during annual estivation. Metabolism, membrane fatty acid composition and passive Na⁺ permeability, sodium pump number and activity were measured to determine their possible role in metabolic depression.

Metabolism studies showed that after an average of 25 days of isolation at 25°C, there was a metabolic depression of about 64% metabolic depression compared with pre-estivation values (range 88-38%).

Passive permeability at 25°C of isolated liver cells was found to be similar in some estivating animals to the control values. However, in two estivating animals with more extreme metabolic depression, sodium content was extremely high compared with control animals (ie. >250 compared with approximately 55 nmol Na⁺/mg P for other animals) indicating that cells flooded with sodium ions. These results suggest complete channel opening and dissipation of Na⁺ gradient during deep estivation. Sodium pump activity (Na⁺,K⁺-ATPase) was found to be similar in both estivating and non-estivating animals in liver, brain, heart and kidney. Sodium pump number was also found not to differ between estivating and non-estivating animals.

The most dramatic changes in membrane phospholipid composition were seen in muscle, where metabolic depression was accompanied by a significant increase in 16:0, 20:4, 22:5/n6, 22:5/n3, and 22:6/n3, and in the unsaturation index, and a decrease in the ω9 fatty acids such as 18:1/n9. From these results, one would expect an increase in membrane fluidity.

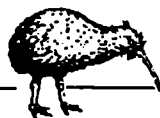
Surprisingly, these results suggest that membrane composition tends to become more unsaturated during metabolic depression, and these changes are seen most in muscle. Sodium pump number and potential activity are retained in metabolic depression but channel opening and sodium pump arrest remain possible for animals in deeper estivational states.

Sodium turnover in two land crabs, *Gecarcoidea natalis* and *Birgus latro* in rain forest on Christmas Island, Indian Ocean.

Peter Greenaway

School of Biological Science, UNSW, Sydney.

Terrestrial crabs have adapted well to the changed requirements of salt balance in the terrestrial vis the aquatic habitat. Control has been switched from the uptake side to output by development of a system for regulating salt loss in excretory fluid. Under laboratory conditions this permits very low rates of loss of major ions and this report will consider the performance of the system under natural conditions and assess how near to the limits of their ion regulatory ability terrestrial crabs live. Data will be presented for sodium turnover in *Gecarcoidea natalis* and *Birgus latro* measured in the field on Christmas Island. In *Gecarcoidea*, the mean Na flux (2-4 mmol.kg⁻¹.d⁻¹) more than 100x that measured under laboratory conditions. Na flux in *Birgus* was higher still (6.5 mmol.kg⁻¹.d⁻¹), again several hundred times greater than measured output in excretory fluid of animals held under laboratory conditions. The sodium flux will be discussed in relation to intake of salts in the diet, chiefly dead leaves in *Gecarcoidea* and fruit in *Birgus*.



Uric acid metabolism in *Gecarcoidea natalis*

Stuart Linton and Peter Greenaway

School of Biological Science, UNSW

The terrestrial herbivorous land crab *Gecarcoidea natalis* like other species of land crabs accumulates large internal purine deposits. These are commonly thought to be extracellular (in the haemolymph) but their physical position, the purines present, their origin and role are essentially unknown. In this study light and transmission electron microscopy have been used to examine the anatomy of the deposits in *G. natalis*. Urate was intracellular in spongy connective tissue throughout the body. Within a cell it existed as numerous membrane bound crystals 1-3 μm diameter. The urate cells lacked obvious organelles (e.g. mitochondria, golgi bodies, RER and peroxisomes) suggesting their function was principally storage and that the urate was synthesised elsewhere. Analysis of extracted purines by HPLC indicated that uric acid was the major component (85% of total purine). The other 15% consisted of hypoxanthine, guanine and xanthine. Nitrogen balance experiments were conducted on groups of animals fed high and low nitrogen diets. After six weeks the total urate content of the high nitrogen group ($116 \pm 45 \mu\text{mol.g}^{-1}$) was significantly greater than that of the low nitrogen group ($72 \pm 40 \mu\text{mol urate.g dry wt}^{-1}$) and animals assayed at the beginning of the experiment ($48 \pm 49.97 \mu\text{mol urate.g dry wt}^{-1}$ ($n=10$)). The metabolites for the accumulated urate originated from dietary intake. The maximum contribution that dietary purine could make to the urate accumulated over the six weeks, by the high nitrogen group, was 0.04%. Hence urate accumulation was not explained by accumulation of dietary purine and by default the urate was synthesised *de novo*. This "voluntary" synthesis indicates that the urate deposits have a metabolic function.

Mechanoreception in the snout of the echidna (*Tachyglossus aculeatus*)

Ed Gregory¹, Ainsley Iggo² and Uwe Proske¹

¹Department of Physiology, Monash University, ²Department of Preclinical Veterinary Sciences, University of Edinburgh

The echidna, one of the three extant monotreme species, is a terrestrial animal living on a diet of ants and other small invertebrates, which are probed for with the snout. The skin of the snout contains an array of simple and morphologically specialised sensory nerve endings, some being similar to types found in other mammals and some organised into sensory organs unique to monotremes, notably 'pushrods' and 'gland duct receptors' (Andres et al. 1991). Gland duct receptors are believed to contain the electroreceptors responsible for the echidna's ability to detect weak electric fields (Gregory et al. 1989).

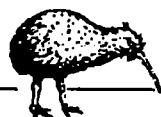
The present study examines the functional properties of mechanoreceptors in the snout, and extends previous work on the subject (Iggo et al. 1985). Afferent responses from 44 units were recorded in filaments dissected from the infra-orbital nerve in 4 animals anaesthetised with chloralose (40 mg/kg, i.v.), after induction with a mixture of ketamine (6 mg/kg) and xylazine (0.5 mg/kg), i.m.

More than half (59%) of the receptors showed slowly adapting responses to mechanical stimulation of the snout. Two categories could be recognised, a type I response characterised by irregular inter-impulse intervals and a type II with regular intervals. In other species, Merkel cells have been shown to generate a Type I response, and in the echidna, Merkel cells are found at the base of pushrods. The remaining receptors were all rapidly adapting, and of two types. One type exhibited a high sensitivity to vibratory stimuli, and showed entrained, 1:1 responses at frequencies as high as 1,000 Hz, a characteristic of Pacinian corpuscles. In the echidna, Pacinian-like lamellated corpuscles are prominent at the base of pushrods and are also found in isolation in the dermis. The four receptor categories were not distinguishable by afferent conduction velocity, threshold for mechanical stimulation, or the size and position of receptive fields.

Andres, K.H., von Düring, M., Iggo, A. & Proske, U. (1991). *Anat. Embryol.*, 184, 371-393.

Gregory, J.E., Iggo, A., McIntyre, A.K. & Proske, U. (1989) *J. Physiol.*, 414, 521-538.

Iggo, A., McIntyre, A.K. & Proske, U. (1985) *Proc. Roy. Soc. Lond. B*, 223, 261-277.



Body temperatures of free-ranging wild dromedaries, *Camelus dromedarius*, in winter and summer, and deprived of water: a preliminary study.

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2 Department of Zoology, The University of Queensland, Brisbane, Queensland 4072, Australia

Knut Schmidt-Nielson's pioneering work on dromedary camels in the 1950's. It showed that, by starting each hot, waterless day cooler than normal and letting temperature rise rather than using water for evaporative cooling, dromedaries without abundant water exhibited striking diurnal cycles in body temperature, from 34-41°C, a daily difference of up to 7°C. This work was undertaken on captive animals, without access to shade and unable to exercise much behavioural influence over their heat exchange. In an extension of behavioural and ecological studies of wild camels in Central Australia (by Döriges and Heucke), we sought to extend Schmidt-Nielson's observations by monitoring body temperatures in free-ranging individuals. All animals showed small but conspicuous diurnal cycles in body temperature; approximately 1.0°C in winter, 1.5°C in summer and up to 2.3°C in summer animals deprived of water. Animals were active in the morning, sought shade during the hottest part of the day and were active (foraging, etc.) in the late afternoon and into the first half of the night, when maximum values of T_B occurred. Rest in the second half of the night, combined with cooler T_A , led to lowering of T_B , reaching a minimum soon after dawn. Deprived of water, the animals sought shade two hours earlier, each day, changed their grazing area to focus on a more succulent species of plant, started each day cooler and tolerated a greater rise in T_B than was seen in well-watered individuals. While, all of the elements described by Schmidt-Nielson appear to be present, they are very much moderated by behavioural compensations. Further work is planned.

The Role of Intrinsic Factors, Extrinsic Effectors and Energy-Consuming Processes in Metabolic Depression.

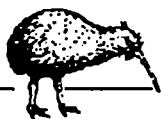
M Guppy¹, C J Fuery¹, J E Flanigan¹, S Pedler¹ and P C Withers²

¹Department of Biochemistry, ²Department of Zoology, University of Western Australia

Metabolic depression is a widespread phenomenon (e.g. hibernation and dormancy), and one that is vital for the survival of many animal species during periods of environmental stress. In fact, humans and common laboratory animals such as rats and mice can be considered exceptions in this regard. The phenomenon of metabolic depression has been fairly well characterised, but many questions remain. Three of the more fundamental ones are: (1) Is metabolic depression an intrinsic cell property, or is an impinging cellular signal required to depress metabolism? (2) What energy-utilizing processes are turned down to effect the depression? (3) What are the mechanisms by which these processes are turned down? We have examined metabolic depression in an invertebrate and a vertebrate animal (snails and desert frogs) during aestivation, and we have developed *in vitro* tissue models of metabolic depression for each of these animals.

We have shown that aestivation in the Australian desert frog *Neobatrachus centralis* is accompanied by an *in vivo* metabolic depression of 77%. Using an *in vitro* liver slice preparation, we have measured an *in vitro* metabolic depression in liver of 55% with a concomitant 67% decrease in the rate of protein synthesis. The decrease in protein synthesis in liver slices accounts for 52% of the metabolic depression of the tissue, but only 4.9% of the metabolic depression of the whole animal.

The metabolic rate of the snail is depressed by 84% *in vivo* within 4 weeks of onset, and this metabolic depression is accompanied by a decrease in haemolymph PO_2 and pH, and an increase in haemolymph PCO_2 . The mantle preparation from estivating animals shows a stable *in vitro* metabolic depression of 48%. The sensitivity of mantle metabolism to PO_2 and pH accounts for 70% of the total metabolic depression, and additional intrinsic effectors contribute a further 30%.



Plasma glucocorticoid concentrations in free-ranging platypus (*Ornithorhynchus anatinus*): seasonal patterns.

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Blood samples were collected from 54 adult female and 50 adult male platypuses captured in gill nets in Victoria between February 1989 and December 1992. All samples were collected within 15 minutes of the animals becoming entangled in the net. There was a clear seasonal pattern in plasma glucocorticoid concentrations in females. Glucocorticoid concentrations were lowest (80-146 pmol/ml) in samples collected between October and July, rose during August (mean \pm SE; 171 \pm 39 pmol/ml) and peaked in September (mean \pm SE; 327 \pm 69 pmol/ml). The peak in plasma glucocorticoid concentration coincided with the period during which the majority of captured females were ovulating (Handasyde *et al.* 1992) and hence with the peak of mating activity. In males, plasma glucocorticoid concentrations were more variable, being relatively low (36-214 pmol/ml) in samples collected between October and June, peaking in July (mean \pm SE; 394 \pm 78 pmol/ml) and remaining moderately high during August and September (245-246 pmol/ml). The peak in plasma glucocorticoid concentrations in males, just prior to the commencement of the breeding season, may be related to intense male-male competition that occurs at this time.

Handasyde, K.A., McDonald, I.R. and Evans, B.K. (1992) Seasonal changes in plasma concentrations of progesterone in free-ranging platypus (*Ornithorhynchus anatinus*). pp 75-79 in Platypus and echidnas. ed. M.L.Augee, Royal Zool. Soc. N.S.W.

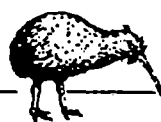
LIMITING FACTORS FOR TRANSIENT pH REGULATION IN MUSCLE TISSUES: PERFUSION AND BUFFERING CHARACTERISTICS

N. Heisler

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The elimination kinetics of surplus H⁺ ions from muscle tissues after burst anaerobic activity are reported to be extremely variable even when referred to the corresponding diffusive elimination of lactate. The efflux half time ratios of lactate/H⁺ range from 0.5 to 80 depending on species and experimental approach, with the lowest values determined in isolated Ringer-perfused or -superfused muscles and the highest values measured in isolated Ringer-suspended thin layer muscle preparations like rat diaphragm and frog sartorius. The isolated blood-perfused dog gastrocnemius muscle exhibits efflux time ratios between these two extremes (1 to 8), which are highly correlated with the rate of muscle perfusion. These data suggest that any transmembrane transfer of H⁺ ions in excess to the amount eliminated from the interstitial space by the perfusate leads to a new pH equilibrium (at a lower level) between intracellular and interstitial space, suspending the driving force for further transmembrane transfer of H⁺ ions.

This notion was closer analyzed on the basis of a four-compartment transfer model utilizing effective intra- and extracellular buffer values, perfusate flow, membrane transfer rates, intracellular/extracellular pH relationships and further parameters. The theoretical model closely resembles and explains the contrasting experimental results, indicating that the buffer capacity (volume x buffer value) of the available extracellular fluid (interstitium + perfusate) is the limiting factor for the rate of H⁺ elimination from muscle cells. The membrane-specific transfer capacity for H⁺ ions, measured in Ringer-suspended thin layer muscle preparations, is accordingly attained in perfused preparations only at blood flow rates > 1.5 l/(min · kg muscle tissue), and, due to the poor closed-system bicarbonate buffering in tissues, at even much higher flow rates of nonbicarbonate buffer-free Ringer solution. Since perfusion rates as high as that are hardly ever attained, the elimination of H⁺ ions from muscle cells has to be considered as largely equilibrium- and perfusion-limited.



**Aspects of the respiratory biology of two New Zealand intertidal fishes,
Acanthoclinus fuscus and *Forsterygion* sp.**

Jonathan V Hill, William Davison and Islay D Marsden
Department of Zoology, University of Canterbury

Aerial and aquatic $\dot{V}O_2$ measurements of *Acanthoclinus fuscus* and *Forsterygion* sp. showed that *A. fuscus* weighing up to 25g obtained oxygen from air and water at similar rates, whereas the ratio of aerial to aquatic $\dot{V}O_2$ for *F. sp.* was mass dependent. The Q_{10} for aquatic respiration of *A. fuscus* and *F. sp.* was found to be 2.3 and 2.5 respectively. Aerial $\dot{V}O_2$ measurements showed that *A. fuscus* could continue to increase its aerial $\dot{V}O_2$ up to at least 25°C. In contrast the aerial $\dot{V}O_2$ of *F. sp.* increased only between 5°C and 15°C and decreased between 20°C and 25°C. The P_{crit} of both *A. fuscus* and *F. sp.* were found to be about 35 torr. Emergence in response to hypoxia occurred between 0 and 16 torr which suggested active emergence was an oxykinetic response and not a behaviour.

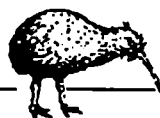
Does Chromatic Aberration Limit Visual Resolution in the Fish?

W.S. Jagger

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Australia

Optical resolution in an eye is degraded by aberrations classified according to their optical behaviour. An appreciation of the design and development of an eye requires knowledge of their relative magnitudes. The trout lens is afflicted with chromatic aberration, and with irregular aberrations present in monochromatic light. Chromatic aberration is a result of the strong dispersion (variation of refractive index with wavelength) of the fish lens material, and as a result only one wavelength at a time can be in focus. For the image of a point object in sunlight, the unfocussed images at other wavelengths are superimposed upon the one sharp image, degrading the total image. These unfocussed disks are relatively large because of the short focal length and large aperture of the lens. Given a lens of a certain aperture and focal length, the chromatic aberration is fixed.

The question arises whether chromatic aberration imposes a limit upon resolution of the fish lens and eye, or whether the monochromatic aberrations are limiting. Calculations and measurements of the modulation transfer function (MTF), which describes the ability of the lens to transmit information, are useful in answering this question. The MTF calculated for chromatic aberration alone is compared with measurements of the MTF in white and monochromatic light. Chromatic aberration is shown to be not limiting. Optical resolution in the fish is therefore a function of lens fine structure, which causes the irregular monochromatic aberrations. The resolution measured is matched to the spacing of the retinal cone mosaic.



The potential for matrotrophy in the viviparous skink, *Niveoscincus metallicus* from Tasmania.

Susan M. Jones and Roy Swain

Department of Zoology, University of Tasmania

In *N. metallicus*, as in most viviparous squamate reptiles, nutrition of the young during gestation is primarily lecithotrophic but the presence of both a yolk-sac placenta and an allantoplacenta suggests the potential for a matrotrophic input into embryonic growth, presumably towards the end of gestation as the yolk is consumed. The relative importance of lecithotrophic and matrotrophic contributions to embryonic growth and development has never been investigated. We assessed the maternal transfer of nutrients in *N. metallicus* by measuring the time course of transfer of tritiated leucine across the placenta at Stages 30 to 40 of gestation, as in Yaron (1977).

Transfer ratios were comparable with those of Yaron (1977). Leucine transfer occurred into embryo, amniotic fluid and yolk, being highest into the embryo. High levels of leucine in the amniotic fluid presumably reflected transfer across the allantoplacenta. Incorporation into the yolk suggests that this may be an additional transfer route.

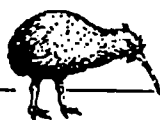
Nutrient transfer occurred throughout the final third of gestation, not only during the last two weeks when the yolk has been consumed. This suggests that maternal input to embryonic nutrition during gestation is significant and may indicate a means of supplementing less-than adequate yolk reserves.

Isolation and characterization of the vasopressin-like gene sequence in the lobster *Jasus edwardsii*.

Jenny Khoo and F.Y.T. Sin

Department of Zoology, University of Canterbury.

The x-organ sinus gland complex in the crustacean eyestalk is an important source for several neuropeptides, among which is the moult-inhibiting hormone (MIH). Previous immunocytochemical studies have shown that the MIH is related to the vasopressin family. Using primers based on the rat vasopressin gene, we were able to amplify by polymerase chain reaction (PCR), a 947 bp fragment from lobster DNA. Using this PCR product as a probe, three different cDNA clones were isolated: peJK1, peJK2 and peJK3. *In situ* hybridization studies and northern blot analysis showed that peJK2 and peJK3 were predominantly expressed in the eyestalk, while peJK1 was expressed in epithelial, eyestalk, heart, hepatopancreas, and muscle tissue of the lobster. Sequence analysis of the cDNA clones showed 96% homology between peJK2 and peJK3. Between 44-51% homology was observed when compared to published MIH sequences from three other species. The biological activity of these encoded peptides has not been investigated.



Oxygen requirements of developing eggs of intertidal crabs

N. Leelapiyanart and H.H. Taylor

Department of Zoology, University of Canterbury, Christchurch

Ovigerous mid-shore crabs, *Heterozius rotundifrons*, carry several hundred large yolky eggs (volume increasing from about 240 to 270 nL). Development time is about 6 months. In contrast, the high shore crab *Cyclograpsus lavauxi* carries several thousand eggs, one twentieth of the volume (10-20 nL), which develop in about two months.

Weight-specific oxygen consumption by inactive, non-ovigerous females of *H. rotundifrons*, at 15°C, in air, was similar to the rate in seawater. In non-ovigerous *C. lavauxi*, oxygen consumption in sea water was slightly higher than in *H. rotundifrons*. Aerial oxygen consumption of non-ovigerous *C. lavauxi* was about double that in seawater. The presence of developing eggs increased the overall weight-specific oxygen consumption (eggs plus crab) in both species and both media. The increase was similar in magnitude in each case and much greater for late stage ovigerous crabs (about 2.4 times the non-ovigerous rate in the case of *H. rotundifrons*).

Weight-specific oxygen consumptions of the separated eggs of *H. rotundifrons* and *C. lavauxi* were also measured in seawater at 15°C. Oxygen consumption increased with development of the eggs, most steeply towards the end. The total cost of development of a single egg of *H. rotundifrons*, in terms of total oxygen consumed, was about 20 times higher than that of *C. lavauxi* (1.517 and 0.077 $\mu\text{mol O}_2$ respectively).

Weight specific oxygen consumption of ovigerous females without their embryos was calculated by subtracting the estimated oxygen consumption of the egg mass from the total oxygen consumed by ovigerous females. Metabolism of the eggs accounts almost completely for the difference (ie carrying eggs only slightly, and non-significantly, increased the mean oxygen consumption of the adult). Despite the enormous difference in body mass of eggs and adults ($> 10^4$ and 10^6 times), weight specific oxygen consumption in water of the early eggs (blastula) and adults of both species were all similar. Metabolic rates were about 10-13 times higher in late stage eggs. The normal scaling of metabolic rate with body mass clearly does not apply to developing crab eggs.

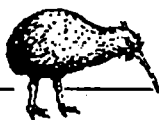
Energy Substrate Utilization by the Brush Tailed Possum (*Trichosorus vulpecula*)

Michael Legge, Bernie McLeod*, Patsy Mason, Janet Crawford* and Grant Shackell*
Department of Biochemistry, University of Otago, PO BOX 56, New Zealand and
AgResearch, Agricultural Research Centre, Invermay, New Zealand*

As a consequence of failure to establish reproductive tract epithelial cells in standard culture media we have attempted to define optimum tissue culture conditions for possum tissue. To define the energy requirements, we have investigated the utilization of three energy substrates in live animals using intravenous tolerance tests for glucose, alanine and propionic acid. Female and male animals had jugular vein catheters inserted 24 hours before testing. Animals were fasted overnight and glucose (1g/kg), alanine (250mg/kg) or propionic acid (125mg/kg) were given as a single dose. Blood samples were collected every 10 minutes prior to and for 60 minutes after metabolite administration then every 15 minutes for a further two hours. For each tolerance test blood glucose, lactate and B-hydroxybutyric acid were analysed.

The response to glucose gave peak glucose concentrations at 20 minutes post-dose with plasma concentrations in males being consistently higher ($p < 0.001$) than in females. However, female animals had higher blood lactate and B-hydroxybutyrate values during the test. Alanine tolerance tests also demonstrated an increased male blood glucose response compared with the female animals. However lactate and B-hydroxybutyrate showed little difference between the sexes. There was no glucose response to the propionic acid tolerance tests. Whilst female animals had slightly higher blood lactate and B-hydroxybutyrate concentrations, neither groups demonstrated a significant response to each other.

These results suggest wide differences in energy substrate metabolism between possum and eutherian mammals.



Ecophysiology of burrow-nesting in the Rainbow bee-cater (*Merops ornatus*)

Alan Lill

Department of Ecology & Evolutionary Biology, Monash University

Burrow-nesting may provide protection from extreme weather conditions and predation, but also necessitate physiological adaptations to cope with the unusual nest atmosphere.

Rainbowbirds in Victoria nested in chambers up to 93 cm below ground entered via a metre-long tunnel. Ambient temperature (T_a) varied diurnally by up to 17°C, but chamber air temperature (T_c) by < 2°C; mean T_c (19°C) was 3°C above mean T_a . This thermal buffering may permit the low mean daytime incubation constancy of 54%.

Chambers were permanently saturated with water vapour and the water vapour pressure gradient across the eggshell was consequently shallow. However, a standard 15% fractional water vapour loss from the egg during incubation was achieved because, although shell conductance was as predicted for a 4g egg, the incubation period (IP) was 53% longer than expected for an egg of this mass. The O₂ fraction of scrubbed chamber air ranged as low as 15.8%, but mean values were only 1-2% below ambient level. The mean PIP embryonic O₂ consumption rate (62 ml d) accorded with allometric predictions from egg mass.

Rainbowbirds developed very slowly. The IP was protracted, but the hatchling exhibited a fairly standard level of maturity, indicating a slow embryonic growth rate. The magnitude of the growth constant K (0.266) for nestlings indicated that their growth rate was slow. The nestling period was very protracted, possibly to facilitate development of muscles mature enough to permit the efficient flight at fledging needed for aerial insectivory.

Are stress responses related to social rank in the grey duck (*Anas superciliosa*) and the laying hen (*Gallus domesticus*)?

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Department of Physiology and Anatomy, Massey University, Palmerston North, NEW ZEALAND

Previously, some studies on mammals have found relationships between plasma levels of cortisol in undisturbed animals and social rank, or between the magnitude of a stress-induced rise in plasma cortisol and social rank. In this study, we investigated the relationship between social rank and the magnitude of stress responses in the native grey duck (*Anas superciliosa*) and the laying hen (*Gallus domesticus*). Both species have distinct hierarchies in natural and captive conditions. The dominance hierarchies of two groups of five birds of each species were determined by behavioural observations and tests of dominance relationships between pairs of birds. The two groups were then combined to initiate a period of social stress and the new hierarchies determined in the same way. The stress response of each bird was defined by the measurement of plasma corticosterone levels in blood samples collected when the bird was first picked up and at 15 minutes and 40 minutes later. Blood samples were collected on days -6, 1, 7 and 26 relative to the day when the groups were combined. Plasma corticosterone levels were elevated at 15 minutes in all birds, whereas after 40 minutes plasma corticosterone had decreased in some birds but not in others. The area under the plasma corticosterone versus time curve was calculated as a measure of total corticosterone secretion over the sampling period. There was no direct relationship between the area under the curve and social rank of the birds. However, middle ranked birds tended to have the greatest area. These results indicate that the stress response may be greatest in birds ranked in the middle of a social hierarchy and hence with most labile social position.



**Changes of sex steroid hormone profiles during artificial maturation of female
New Zealand longfinned eels (*Anguilla dieffenbachii*)**

P. Mark Lokman and Graham Young

Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand

European and Japanese eels, which are the most-studied anguillids, are relatively immature at the onset of the spawning migration. As a result, data on the reproductive physiology of freshwater eels is lacking. Artificial spawning induction is one of the experimental approaches used to investigate gonadal development, but application of this technique is impeded by our lack of knowledge of eel gametogenesis in the wild. Since gonadal development at the onset of the spawning migration is considerably more advanced in the New Zealand longfinned eel (*Anguilla dieffenbachii*) than in most other eel species, we have recently been able to determine sex steroid profiles of early to mid-vitellogenic eels from a natural population (unpublished results). In the present study, we describe how sex steroid profiles change during artificially induced gonadal development and how these profiles relate to those from eels from the wild. Ten migratory female longfins (1.1-3.9 kg) were divided between two groups and repeatedly injected with saline or salmon pituitary homogenate (SPH). Blood samples and ovarian biopsies were collected and changes in sex steroid profiles and oocyte development monitored. Upon reaching the migratory nucleus stage, final oocyte maturation and ovulation were induced with SPH and 17 α -hydroxyprogesterone. All SPH-injected females reached maturity after 33-55 days of treatment. Plasma estradiol-17 β levels increased during treatment from 0.5 ng.ml⁻¹ at the start of the experiment to more than 2 ng.ml⁻¹ during vitellogenesis. Treatment also induced increases in plasma 11-ketotestosterone (generally considered a male-specific hormone) from 0.3 ng.ml⁻¹ at the start to 1.3-2.0 ng.ml⁻¹ during the remainder of the experiment. Plasma levels of several other sex steroid hormones will be reported. Results will be discussed in relation to sex steroid profiles of eels from the wild and those of artificially matured European and Japanese eels.

**Seasonal changes in the renal morphology of *Antechinus stuartii*
(Marsupialia: Dasyuridae)**

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Department of Physiology, University of New England, Armidale, N.S.W. 2351

The renal morphology of *Antechinus stuartii* was examined in males and females in February, May, July and August. Relative medullary thickness, percentage medullary thickness and medullary thickness were not different between seasons or sexes. Both outer cortex and juxtamedullary glomerular volumes increased in July and August in males and this coincided with a reduction in glomerular number per mm² of cortex. In general tubular diameters increased in males from July and August, but not in females. The same was true of cellular volumes from the same sections of the nephron. The renal morphological changes correlate with the life history and endocrine changes of *A. stuartii*.



A novel animal model for investigating the aetiology of ovarian tumours ?

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The Inverdale gene (Fec-X^I) is a major prolificacy gene that effects ovarian function in sheep. Heterozygous carriers have enhanced antral follicle development and an increased ovulation rate, but homozygous ewes display ovarian hypoplasia and a complete absence of antral follicles. The ovarian hormones oestradiol and inhibin, produced by large antral follicles, normally control gonadotrophin secretion via a negative feedback system. Ovaries of homozygous Inverdale ewes, do not produce these hormones, so gonadotrophin secretion is unconstrained. In women, the peak incidence of ovarian cancer occurs after the menopause when antral follicle development fails and gonadotrophin levels increase. It is suggested that elevated gonadotrophin concentrations predispose to the formation of ovarian tumours at this time. Some classes of ovarian tumour are known to secrete inhibin, and this is often used as a diagnostic tool.

Laparoscopic observation has shown that large tumour-like structures periodically develop within the ovaries of a proportion of homozygous Inverdale ewes. These structures are sometimes associated with extremely high plasma concentrations of inhibin. We have screened homozygous ewes over a two-year period, for the presence of ovarian structures and for aberrant hormone secretion patterns. Ovarian structures were recorded in about one-third of animals. Their morphology varied, but they could be classified as fluid-filled cysts, solid vascular or avascular tissue masses or haemorrhagic structures. Their appearance was sometimes associated with high plasma concentrations of inhibin, which in some instances remained elevated for several months or increased before ovarian structures became macroscopically visible. We suggest this animal may contribute to an increased understanding of the early development of ovarian tumours.

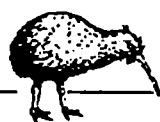
Natriuretic Peptide Receptors in the Kidney of the Toad, *Bufo marinus*

Stuart Meier and John A. Donald
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The family of natriuretic peptides (NPs) has been shown to contain at least four structurally different peptides: ANP (atrial NP), BNP (brain NP), C-type NP, and urodilatin. Together, these peptides reduce hypertension associated with hypervolemia by facilitating vasodilatation and renal diuresis and natriuresis. They also antagonise the actions of vasopressin and the renin-angiotensin system, thus reducing the reabsorption of sodium and water in the kidney.

In mammals, radio-ligand binding studies in renal tissue have identified NP binding in the glomerulus, ascending loop of Henle, and collecting ducts. Amphibians offer a unique model for studies of blood volume and osmoregulation since exposure to fresh water results in a net water gain. NPs, therefore, could be critical in maintaining volume and osmotic homeostasis. The present study focuses on the regulation of kidney function by NPs in the anuran amphibian, *Bufo marinus*, by determining the distribution of ¹²⁵I-ANP (termed NP binding here) binding sites in this tissue. Non-specific binding was determined by using unlabelled ANP and the specific NPR-C ligand C-ANF as competitors. Specific binding sites were observed on glomeruli and on blood vessels that could be glomerular afferent arterioles. Specific binding was observed over the area of the kidney tubules, however, the relationship of this binding with specific segments of the kidney tubule has not been determined. C-ANF displaced most, but not all binding, which suggests that two types of NP binding sites are present.

These data suggest that NPs could be important regulators of glomerular function and renal blood flow. Their role in tubular regulation remains to be determined. These studies are being extended to the urinary bladder since this tissue is comparable in nature to the collecting duct. In addition, similar studies are being performed to determine the distribution of AVT binding in the kidney to enable comparison with the NP binding pattern.



The evolution of sleep in mammals: the significance of the dreams of monotremes

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Sleep like behaviour occurs widely throughout the animal kingdom. While there have been suggestions that some reptiles show EEG patterns corresponding to those of sleeping mammals, "true" sleep seems to be restricted to endotherms and it has been argued (Allison & Van Twyver, 1970, *Nat Hist* 79, 56-65) that sleep evolved in parallel with endothermy as a mechanism for reducing energy expenditure during inactive periods. On the basis of electrophysiological criteria, sleep in mammals can be divided into two distinct phases: rapid-eye-movement sleep (REM), and slow wave sleep (SWS). In a number of aspects of its anatomy and physiology, the echidna shows a mixture of reptilian and mammalian characters and thus the status of sleep in the echidna becomes very significant. Allison et al (1972, *Arch Ital Biol*, 110,145-184) made an electrophysiological study of sleep and wakefulness in 5 echidnas and found no clear evidence of REM. From this they concluded that SWS and REM arose sequentially in the course of mammalian evolution, and that SWS appeared first, probably in relation to the development of endothermy, while REM appeared later in the early therian ancestors of marsupial and placental mammals. Using subdermal needle electrodes we recorded electrooculogram (EOG), neck muscle electromyogram (EMG) and EEG activity continuously from 4 unrestrained echidnas exposed to ambient temperatures ranging from 15-28°C. These recordings showed the cyclic occurrence of typical mammalian episodes of REM sleep, lasting up to 20 minutes, during their major nocturnal sleep periods at all ambient temperatures. These REM periods are characterised by a desynchronised EEG, REMs, and a tonic EMG at or below prior SWS levels. Phasic EMG bursts occasionally accompany REMs. The presence of REM sleep in the echidna indicates that both REM sleep and SWS had already evolved in earlier mammalian ancestors.

Muscle degeneration in wild emus

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During a study of the structural and metabolic properties of muscles used to power running in the emu (Patak and Baldwin, 1993) we observed that several major muscles including the iliotibialis, gastrocnemius and digital flexors showed signs of degeneration. Half of the (approximately 10) apparently healthy wild birds examined displayed various degrees of muscle breakdown.

Commonly the muscle fibres were loosely packed, circular and swollen in cross section, instead of tightly packed and polygonal. Myosin ATPase and phosphorylase activities were reduced or absent, and the sarcoplasm often showed cracks. Staining for NADH tetrazolium reductase activity revealed mitochondria arranged in whorls around the fibre perimeter. In some fibres the sarcoplasm had shrunk leaving empty myotubes.

Anecdotal reports suggest that myopathic conditions are not uncommon among farmed emus. The results of our study show that this problem is not unique to captive animals.

Patak, A, and Baldwin, J. (1993) *J Exp Biol* 175, 233-249.



Respiratory gas exchange in vertebrates: modeling based on structure and function

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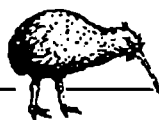
The basic parameters in the analysis of external gas exchange in vertebrates are ventilation, diffusion and perfusion. Gas transfer is determined by the conductances defined as transfer rate per partial pressure difference. The convective (ventilatory and perfusive) conductances are the products of flow and effective solubility. The high effective solubility ratio CO_2/O_2 in water and tissue causes a high ventilatory conductance ratio CO_2/O_2 and a low P_{CO_2} in expired medium and blood. The diffusive conductance (diffusing capacity) is mainly determined by anatomical dimensions (surface area, thickness) and material properties (diffusion coefficient, solubility). A useful model for characterisation of the role of diffusion limitation is the diffusive/perfusive conductance ratio (equilibration index). The anatomical arrangement of medium (gas or water) and blood flows can be represented by four basic models: ventilated pool (mammalian, reptilian, amphibian lungs), cross-current (bird lungs), counter-current (fish gills) and open model (skin). In theory, the highest gas exchange efficiency can be attained by the counter-current model, followed by the cross-current model, but measurements show that the theoretical limits are not reached due to diffusion limitation and heterogeneities. The unequal distribution of ventilation to perfusion in mammalian lungs (\dot{V}/\dot{Q} inequality) and shunt (or venous admixture) are known to be the major sources of gas transfer inefficiency. \dot{V}/\dot{Q} inequality has been shown to be present in bird lungs and reptilian lungs. Various kinds of shunts play a major role in reptiles, amphibians and fishes. The distinction between the effects of diffusion limitation, \dot{V}/\dot{Q} inequalities and shunt is in many cases difficult, particularly in water breathing.

Whey proteins genes of the common brush tailed possum (*Trichosurus vulpecula*)

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Lactation in marsupials is characterised by the presence of two distinct phases. The milk composition is quite different during each phase and major changes in mammary gene expression occur at the switch between them. In the possum the switch occurs around day 110 of lactation and corresponds to the time that the pouch young first leaves the pouch. The major whey proteins of the possum have been separated by 2D electrophoresis, electroblotted and identified by N-terminal amino acid sequence analysis. Two cDNA libraries have been constructed using RNA extracted from mammary tissue collected in early or late lactation. Clones for six of the major whey proteins have been obtained and their DNA base sequences determined. Several genes are expressed throughout lactation. These include β -lactoglobulin, α -lactalbumin, lysozyme and a novel protein with sequence similarities with mouse urinary proteins. Two proteins, transferrin and a possum homologue of the wallaby late lactation protein are expressed only in late lactation whereas a second novel protein which has sequence similarities to a family of protease inhibitors is expressed only in early lactation.



A model for salt gland secretion in the green sea turtle, *Chelonia mydas*.

Richard Reina

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Very little is known about the control of salt gland secretion in sea turtles. *In vivo* and *in vitro* techniques were used to investigate the role of cholinergic and adrenergic stimulation as possible control modifiers of salt gland activity. The *in vivo* technique measured salt gland response to injection of methacholine and adrenaline in salt loaded turtles. *In vitro* techniques included measurement of oxygen consumption of salt gland cells and immunohistochemical localisation of adrenergic nerves. The minimum salt load required to initiate a secretory response was also determined.

There was a dose dependent inhibition of the salt gland *in vivo* by methacholine, with duration of inhibition increasing with dose from 1 to 10 mg.kg⁻¹. In addition, some residual inhibition was evident after secretion had commenced, with the total rate of secretion reduced in comparison to control animals.

Adrenaline also exerted a profound, dose dependent inhibitory influence on salt gland activity *in vivo*. The minimum dose effective in inhibiting secretion was 25 µg.kg⁻¹ and the period of inhibition increased with doses up to 500 µg.kg⁻¹. When secretion recovered, it resumed at control rates and did not display any residual inhibition.

Adrenaline did not affect the oxygen consumption rate of salt gland cells *in vitro*, but it did increase the rate of consumption by turtle cardiac cells. Cells from both active and inactive salt glands consumed oxygen at about 15 µl.min⁻¹.g wet weight⁻¹.

The presence of adrenergic nerves within the salt gland was shown by the presence of tyrosine hydroxylase. Adrenergic nerves were found to be present around the main collecting area of secretory fluid, and also running between lobes of the gland.

The minimum salt load necessary to initiate salt gland secretion was between 400 and 600 µmol.100g⁻¹, with an elevation in plasma sodium being detected.

The role of salt loading, adrenergic and cholinergic influences on salt gland activity will be discussed in the context of a model for regulation of salt gland function.

The responses of the avian kidney to a reduction in renal mass

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The right ureter was severed in 2-3 week old cockerels, at the level of the ischiadic artery (between the medial and caudal kidney divisions). Sham operations were performed in the control group. Animals were then sampled at 1, 2, 4, 6, 10 and 14 weeks following the initial surgery. At each of these times, 5 birds were selected for glomerular counts and 5 for histological examination of the kidney tissue. For estimates of numbers and sizes of glomeruli, birds were anaesthetised, a wing vein was cannulated and 0.25% Alcian blue solution in 2.5% mannitol was infused. Birds were sacrificed and kidneys removed and placed in ethanol with ammonium hydroxide. At a later time, kidneys were digested in 20% hydrochloric acid, suspended in water and aliquots placed in a Sedgewick-Rafter cell for counting under a microscope with calibrated eyepiece. Tissue taken for histological examination was processed using standard techniques. The results indicated that new nephrons are formed in this strain up to 5 weeks of age, with subsequent kidney growth accomplished by enlargement of existing nephrons. In the chickens with the severed right ureter, the formation of new nephrons was arrested in both kidneys during the first 2 weeks post-surgery. There was some loss of existing nephrons in the kidney tissue upstream from the ureteral severance (atrophy) with these nephrons being replaced by connective tissue and fat. Two weeks after the surgery, the kidney tissue downstream from the severed ureter, and the contralateral kidney, underwent hyperplasia followed by hypertrophy in order to compensate for the loss of functional renal mass.



The effect of saline loading on renal function and plasma hormone levels in chickens

Alison Leary and Julie Roberts

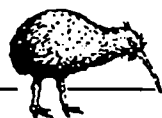
Department of Physiology, University of New England, Armidale, NSW 2351.

The effect of hypertonic saline loading on renal function and plasma hormone levels in chickens was investigated. Birds were anaesthetised and the one carotid artery, one brachial vein and the cloaca cannulated. For the first 60 mins of the experiment, birds were infused with isotonic sodium chloride solution containing inulin and PAH. Then the infusion was changed to hypertonic (1M) sodium chloride containing the same concentrations of the renal function markers and infused for 90 mins. Urine samples were collected every 30 minutes. Blood samples were collected and the plasma reserved to allow for assays of the hormones aldosterone and prolactin, chemical analyses for the renal function markers and analyses for sodium, chloride, potassium and osmolality. Urine samples were analysed for electrolytes and renal function markers. The infusion of the hypertonic sodium chloride solution resulted in significant increases in plasma sodium, chloride and osmolality and a significant decrease in haematocrit. This was accompanied by increased fractional excretion of sodium, potassium and chloride, and increased osmolal output, without any significant change in urine flow rate. In spite of a significant increase in the plasma clearance of para-aminohippuric acid, glomerular filtration rate did not change significantly as the result of the saline loading. Plasma prolactin and aldosterone levels decreased significantly in response to the salt load. The osmotic stress of hypertonic sodium chloride infusion produces physiological changes which may be conflicting. The increases in plasma electrolyte concentrations would tend to stimulate the release of arginine vasotocin (AVT) whereas the volume expansion (evidenced by the decrease in haematocrit) would tend to suppress AVT release. In the present study, the result was that the plasma flow to the kidneys increased but there was no change in glomerular filtration rate.

The development of endothermy in the Tasmanian bettong (*Bettongia gaimardi*)

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Departments of Zoology and Biochemistry*
University of Tasmania. Hobart, Australia.

Marsupials, at birth, are ectothermic and gradually attain the ability to change their metabolic heat production during pouch life. How this process occurs in the bettong has been measured on 13 pouch young from week 1 until 3 weeks after pouch vacation (week 16). Oxygen consumption was measured at 35°C (pouch temperature) and at 22°C. The results at 35°C showed an increase in metabolic rate from week 1 until week 12 when there was a decrease to near adult levels after pouch vacation. At 22°C young bettongs had a lower metabolic rate (compared with measurements made at 35°C) until week 9 after which there was an increase above measurements made at 35°C. Noradrenaline had little effect until week 10 after which the metabolic rate (although measured at 35°C) paralleled the measurements obtained at 22°C. Measurements of total thyroid hormone (T4) at 35°C and 22°C paralleled the changes in oxygen consumption.



Central cardiovascular shunts in the marsupial heart

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Birth represents a period of major cardiorespiratory adaptation and survival of the young depends on a smooth transition from placental gas exchange to gas exchange via the lungs. The success with which this is achieved is dependent in part on structural changes that occur in the cardiovascular system. The structure and timing of closure of central cardiovascular shunts have been comprehensively investigated in eutherians. Marsupials are born at an early stage of development after a relatively short period of gestation. In this study the nature and timing of closure of central cardiovascular shunts have been investigated in eight marsupial species. Light and scanning electron microscopy were used to investigate the structure and the changes in central shunts in perinatal marsupials with gestation periods of between 12.5 and 36.5 days and birth weights ranging between 12.5mg and 740mg. Laboratory mice were used as a eutherian comparison. The neonatal marsupial has two central cardiovascular shunts, a ductus arteriosus and an interatrial communication via a fenestrated septum. The ductus arteriosus occludes soon after birth but the interatrial communication closes over a period of days after birth as a result of tissue proliferation. In one species with a birth weight of 12.5mg two additional shunts, an interventricular communication and incomplete septation of the outflow tract, were found. Marsupials have similar cardiovascular strategies for dealing with the transition from intra-uterine to extra-uterine life. However the central shunts differ in structure and in the timing of closure.

Folliculogenesis in the Brushtail possum (*Trichosurus vulpecula*)

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Gonad differentiation, ovarian development, germ cell proliferation and early folliculogenesis in eutherian mammals, occurs *in utero*. Germ cell numbers peak at about the end of the second trimester and subsequently decrease due to atresia. At birth, the number of germ cells present in the ovaries is approximately 40% of the peak numbers recorded during gestation, meiotic proliferation is completed and the ovaries hold the lifetime supply of oocytes in primordial follicles. The initiation of follicle growth, which begins when oocytes reach the diplotene stage, may occur during foetal life or after birth, depending on species.

In contrast, the marsupial is born at a much earlier stage of development and at parturition the foetus still has indifferent gonads. The ovaries do not begin to differentiate for 3-7 days and meiosis, germ cell proliferation, and early folliculogenesis all occur during early pouch life. However, the intrinsic pattern of germ cell production, namely a rapid increase in numbers followed by a subsequent reduction due to atresia, is the same as in eutherians.

Studies of folliculogenesis in the Brushtail possum have begun to resolve the temporal patterns of ovarian development and folliculogenesis in this species. Follicle growth is initiated at approximately d70, which is about the same time that germ cell numbers peak. Primary follicles first appear at about d100, secondary follicles at about d105 and antral follicles at about d155 after parturition. While the pattern of folliculogenesis appears to be similar among the marsupials, previous reports using different species (Tamar wallaby and Bandicoot) suggest temporal differences.



Entry into daily torpor: time course of metabolic rate and body temperature reduction

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Physiological mechanisms causing reduction of metabolic rate (MR) during torpor in heterothermic endotherms are poorly understood. While the steady-state MR during torpor (TMR) above the set-point (T_{set}) for body temperature (T_b) appears to be well explained by the effect of T_b on metabolic processes, it has been suggested that metabolic inhibition may be involved in the substantial drop of MR during the entry phase. In the present study, we simultaneously measured instantaneous rates of oxygen consumption and T_b of *Sminthopsis macroura* during torpor entry at ambient temperatures (T_a) of 25 and 18 °C (above the T_{set}) and at 10 °C (below the T_{set}). At all T_a s, torpor was initiated by a decrease of MR from active or resting levels, which was followed by a decrease of T_b . At T_a s above the T_{set} , T_b and MR decreased more or less parallel and the reduction from BMR to TMR when both T_b and TMR were minimal can be explained by temperature effects ($Q_{10} \sim 2.8$). However, because the TMR minimum was reached when the T_b was still about 0.7 °C above its minimum, a small fraction of the TMR reduction below BMR during the entry phase cannot be explained by temperature effects and suggests that some additional metabolic inhibition is involved. It is also possible that some of this reduction is due to a shift in RQ. At the T_a below the T_{set} , the pattern of MR and T_b reduction differed from that above the T_{set} . TMR showed an initial undershoot to about 60% of BMR and then increased again to values above the BMR. In contrast, the T_b often showed a steady decline throughout the entire entry phase giving the impression that TMR and T_b are not related. However, the T_b at the point of the TMR undershoot had dropped by about 10 °C thus providing a possible explanation for the TMR undershoot. The following increase of TMR while T_b was still falling is explained by a transient state between largely passive cooling during early entry and physiological thermoregulation as the T_{set} is approached.

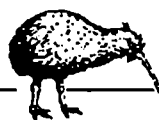
Do some species of *Niveoscincus* (Lacertilia: Scincidae) supplement autumn mating with a second mating in spring?

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Niveoscincus metallicus and *N. ocellatus* are common Tasmanian skinks that are characterized by late autumn mating, followed by winter torpor and spring ovulation. Females consequently store sperm throughout winter. Testes size peaks in summer, followed by maximum plasma testosterone concentrations in autumn coincident with maximal epididymal development. In females plasma estradiol concentrations are significantly elevated during the preovulatory phase (early spring). Histological evidence confirms that autumn matings occur in both species. However, evidence from mating scars suggested that a second mating occurs in at least some females during preovulation in early spring.

This paper presents the results of a preliminary investigation of this possibility. Data are presented for the state of 'sexual readiness' of males caught in spring (willingness to extrude hemipenes and extrude sperm), and the anatomical and histological status of the reproductive tract. Plasma testosterone concentrations are compared in inactive and potentially active males. The implications of a second mating program in these species are considered briefly.



Lipid and protein metabolism in eggs of small skinks, *Morethia* and *Eumeces*

¹Michael B. Thompson, ²James R. Stewart and ¹Kylie J. Russell

¹School of Biological Sciences, University of Sydney, ²Faculty of Science, University of Tulsa

Closed-system respirometry was used to measure rates of carbon dioxide production and oxygen consumption in developing eggs from small skinks. Respiratory exchange ratios were 0.75-0.79 in all species, suggesting that the metabolic substrate consists of approximately equal quantities of protein and lipid. This result contrasts with that for other amniotes where lipid is the main source of metabolic energy. Given the relatively low energy density of protein compared to lipid, this finding raises many puzzling questions. Metabolism of protein creates a problem with production of nitrogenous waste and would require a larger egg to contain the same amount of energy. We are presently exploring these questions further using lipid and protein analyses, and bomb calorimetry.

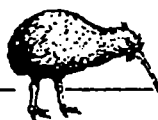
The significance of cardiac hypertrophy and other physiological changes in maturing male rainbow trout (*Oncorhynchus mykiss*)

Helgi Thorarensen and Peter S. Davie

Department of Physiology and Anatomy, Massey University

During reproductive maturation the ventricular mass of rainbow trout males, but not females, increases disproportionately relative to body mass as a result of elevated levels of plasma androgens in the males. The cardiac output (Q) of the enlarged hearts of the males in *in situ* preparations was greater than in females, because the amount of blood ejected with each heart beat (SV) was larger. Steroids also increase haematocrit (Hct) in intact fish and thus elevate the O_2 content of arterial blood. Increases in Q and Hct elevate the O_2 transporting capacity of the cardiovascular system (TO_2). Androgens stimulate growth of the aerobic swimming muscles in trout. We hypothesised that the increased TO_2 and the greater amount of aerobic swimming muscles would improve the swimming performance of the mature males. To test this hypothesis, we compared Q , SV , arterial blood pressure (Pa), Hct and swimming performance, as determined in step increment tests (U_{crit}), in mature male and female trout.

The ventricles of the males were 80% larger than those of same size females. However, contrary to what was observed *in situ* preparations, maximum Q was not significantly different in the sexes. Maximum Pa was 30% higher in the males and maximum power output of male hearts was 40% higher than in the females. SV was 60% higher at rest in the males but it was not significantly different at maximum Q . Therefore, we suggest that the increased ventricle mass in the males is an adaptation to support higher Pa and power output rather than to increase Q . The Hct of the males was 40% higher in the males, suggesting that maximum TO_2 was 60% greater in the males than in the females. Although, TO_2 of the males was increased, their U_{crit} was not different from that of females.



The rhythm of life experienced by the New Zealand freshwater crayfish,
Paranephrops zealandicus

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Department of Zoology, University of Canterbury.

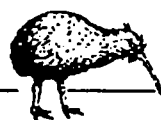
Diel rhythms in behaviour and physiology have been reported for many Crustacea, including New Zealand freshwater crayfish, *Paranephrops zealandicus* and *P. planifrons*. At 18°C, *P. zealandicus* demonstrated significantly more voluntary emersion activity during darkness than during daylight, on their own ($P = 0.0012$) and in pairs ($P = 0.0026$). Crayfish kept in air for 48 hours demonstrated significant pH_a oscillations ($P = 0.0004$), with the dusk pH 0.1 units more alkaline than the dawn pH . In the evening, crayfish settled in water also demonstrate a significantly higher heart beat frequency f_H , ($P = 0.027$), and ventilation frequencies f_R , for the faster ($P = 0.024$) and the slower ($P = 0.009$) scaphognathites. The dusk alkalosis may anticipate a potential acidosis associated with the nocturnal increase in activity and oxygen demand.

Changes in the binding characteristics of natriuretic peptide receptors in the Atlantic hagfish
(*Myxine glutinosa*) correlated with environmental salinity

Tes Toop

School of Biological and Chemical Sciences, Deakin University

Natriuretic peptides (NPs) are involved in salt and water homeostasis in mammals and are implicated in fish osmoregulation. We have characterised natriuretic peptide receptors (NPRs) in the gills and kidneys of the osmoconforming Atlantic hagfish, *Myxine glutinosa*. These organs are fundamental to the maintenance of osmotic status in fishes. The gills contain two types of NPR, a guanylate cyclase-linked receptor with similarity to the NPR-A of mammals that preferentially binds atrial natriuretic peptide (ANP), and a receptor resembling the NPR-C of mammals that binds all NPs. The kidneys appear to contain only the NPR-A-like receptor. We studied the binding properties of NPRs in hagfish exposed for 12 h to 100%, 85%, and 115% sea water (SW) to examine the potential role of NPs in hagfish volume regulation. Competition binding studies for ^{125}I -ANP (I-ANP) sites were performed on either isolated gill membranes ($n = 6$ for each salinity) or on 20 μm tissue sections of the kidney that were subsequently exposed to X-ray film and analysed using grey scale analysis ($n = 3$ for each salinity). Non-radioisotopic competitors ($10^{-12} M - 10^{-6} M$) were rat ANP (rANP), porcine C-type natriuretic peptide (pCNP) and C-ANF, an artificial ring deleted NP analogue that binds NPR-C exclusively. The most consistent changes in salinity-adjusted hagfish were associated with pCNP competition for I-ANP sites. In the control gills (100% SW), 20 nM pCNP competed for 50 % of I-ANP sites, but in high (115% SW) and low (85% SW) salinities, pCNP competed for 50 % of the sites at 0.9 nM and 2 nM respectively. In control kidneys, 300 nM pCNP competed for 50% of I-ANP sites; however, in high and low salinities, 1 nM and 20 nM of pCNP competed for 50 % of sites. The competition of C-ANF for I-ANP sites did not alter in the salinity adjusted animals which indicates that the NPR-C-like receptor is unaffected by salinity changes. 1 nM rANP competed for 50% of sites in control gills; the experimental 50% values were 0.1 nM (115% SW) and 3 nM (85% SW). There were no major differences in the rANP 50% competition values in kidneys, which ranged between 1 - 4 nM. In both experimental salinities, 10 nM of rANP had competed for over 90% of I-ANP sites, in contrast with control kidneys in which I-ANP still bound 40% of the sites. Clearly NPR binding parameters are altered by changes in environmental salinity. These changes are not apparently associated with the NPR-C-like receptor. Whether the changes reflect alterations in binding to the NPR-A-like receptor, or are indicative of the expression of a third type of receptor, is still being determined.



Ammonia/ammonium excretion and whole animal volume changes in the sea star (*Patiriella calcar*) following 24 h of hypo-osmotic and hypo-thermal stress

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The relationship between $\text{NH}_3/\text{NH}_4^+$ excretion and cell volume regulation in marine invertebrates is well recognised; however, the combination of salinity and temperature stresses on the volume regulating ability of such organisms is largely undetermined. The sea star, *Patiriella calcar*, was exposed for 24 hours to hypo-osmotic and hypo-thermal stress in order to examine its volume regulating ability under these combined conditions. As an intertidal rocky shore species found in S.E Australia, *P. calcar* is exposed regularly to seasonal variations in environmental salinity and temperature. Individual sea stars were exposed to a combination of 80% or 100% sea water (SW) and 5°C or 10°C. Individuals were weighed before and after treatment and water samples were analysed for total $\text{NH}_3/\text{NH}_4^+$. Net $\text{NH}_3/\text{NH}_4^+$ efflux of control animals (100% SW, 10°C) was 9.2 ± 0.5 $\mu\text{mol}/100$ g dry mass/h. Net $\text{NH}_3/\text{NH}_4^+$ efflux in experimental groups was significantly higher than in the control group (80% SW, 10°C = 54.2 ± 3.4 $\mu\text{mol}/100$ g dry mass/h; 100% SW, 5°C = 35.0 ± 5.7 $\mu\text{mol}/100$ g dry mass/h; 80% SW, 5°C = 45.0 ± 3.3 $\mu\text{mol}/100$ g dry mass/h). The 24 h wet mass values did not differ from initial values in any group except for sea stars in 100% SW and 5°C which decreased their original mass by 8% (paired t-test, $p = 0.03$). Animals in 80% SW appear to compensate for osmotic water gain within 24 h by increasing their net $\text{NH}_3/\text{NH}_4^+$ efflux, as would be expected. Animals exposed to hypo-thermal and hypo-osmotic stress (80% SW, 5°C) appear equally able to volume regulate within 24 h as those exposed to hypo-osmotic stress alone (80% SW, 10°C). However, animals in 100% SW but 5°C unaccountably increased net $\text{NH}_3/\text{NH}_4^+$ efflux and lost weight. Some of the physiological conditions, therefore, occasioned by hypo-osmotic stress may mask, in dual-stress situations, those physiological conditions correlated with hypo-thermal stress alone. Future time-course studies will address this issue by examining volume regulation and metabolic rates in single-stressed and dual-stressed sea stars.

Intestinal Na^+, K^+ -ATPase activity in chinook salmon: regional differences along the gut and the effect of seawater adaptation

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Teleosts combat dehydration in seawater by absorbing water across the intestine. In the anadromous salmonids, water absorption increases in the posterior region of the gut during the parr-smolt transformation, when developmental changes occur in fresh water which prepare juveniles for life at sea. Previous studies have shown that water absorption across the gut is coupled to the ion translocating Na^+, K^+ -ATPase (sodium pump) located on the basolateral membrane of intestinal epithelial cells. To better understand the mechanism behind seawater-adaptive changes in the gut, we measured the activity of Na^+, K^+ -ATPase in three gut regions (pyloric caeca, middle, and posterior intestine) of chinook salmon (*Oncorhynchus tshawytscha*) adapted to fresh water or seawater. Since elevated Na^+, K^+ -ATPase of the gill is a characteristic of seawater-adapted salmonids, we also measured concurrent changes in activity of this enzyme. As expected, gill Na^+, K^+ -ATPase activity was higher in seawater-adapted fish than of those in freshwater. Na^+, K^+ -ATPase activity was highest in pyloric caeca relative to the middle or posterior intestine, regardless of whether the fish were in fresh water or seawater. Our most important findings were that in both pyloric caeca and posterior intestine, Na^+, K^+ -ATPase activity was higher in seawater compared to fresh water. These results suggest the pyloric caeca may be a major site of water uptake and play a role in osmoregulation, in addition to its well known role in nutrient absorption. Furthermore, an increase in sodium pump activity is probably the mechanism by which water absorption increases in the posterior intestine, and possibly the pyloric caeca, during the parr-smolt transformation.



The effects of captivity and of cortisol *in vitro* on estradiol-17 β production in greenbone (*Odax pullus*)

Rob.T.Wass and Graham Young

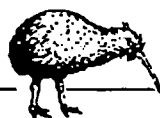
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It is well known that the stress of captivity can have profound negative effects on reproduction in teleost fish. In an effort to ascertain the aquaculture potential of a marine teleost, the greenbone, we held fish in captivity for 2-4 months with the result that oocyte development was arrested in early to mid-vitellogenesis. Cortisol (F) levels in these fish were highly variable but often greater than 50 ng/ml. Initially, plasma sex steroid levels in greenbone sampled within 30 minutes of being gill-netted were 1.5-3.0 ng/ml estradiol-17 β (E2), 0.3-1.0 ng/ml testosterone (T) and 0.1-0.7 ng/ml androstenedione (AD). Plasma E2 levels declined rapidly after initial capture and remained at less than 0.2 ng/ml. Two weeks after capture androgen levels had returned to levels seen immediately after capture, with a tendency for increased levels during the remaining period of sampling. Because aromatizable substrate (AD, T) was similar or higher than that seen in wild fish, we investigated the possibility that arrested oocyte development may be a result of stress (mediated by cortisol) suppressing aromatase activity directly at the level of the ovarian follicle. We therefore incubated ovarian follicles from three freshly wild-caught, vitellogenic greenbone females with or without gonadotropin or steroid substrates in the absence or presence of F. Basal production of E2 was generally low and variable and was suppressed by 35% in the presence of F. Gonadotropin-stimulated and substrate-supported E2 production was generally suppressed in the presence of F, although the extent of the suppression varied and may depend on the stage of vitellogenesis. This preliminary study suggests that F may directly mediate the effects of stress at the level of the ovary in the greenbone at least during some stages of vitellogenesis.

Effects of shore level and temperature on aquatic and aerial respiration of the mussel *Perna canaliculus*.

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Aerial and aquatic rates of oxygen uptake were measured for *P. canaliculus* collected from mid and low tidal levels at Taylors Mistake, Canterbury during summer (December to February) and winter (May to July). Weight specific oxygen uptake depended upon body size and consistent with other species of mussels, the aquatic rates exceeded the aerial rates. Mussels demonstrated a lateral seasonal shift in the rate-temperature curves for both aerial and aquatic respiration. Aquatic respiration increased with higher exposure temperature, from 5 to 15 $^{\circ}$ C in winter and 10 to 20 $^{\circ}$ in summer. In contrast, aerial oxygen uptake decreased at the highest exposure temperature in winter and summer. For each exposure temperature, rates of oxygen uptake were similar for mussels collected from mid and low tidal levels. This suggests that *P. canaliculus* does not compensate for tidal level by adjusting its rates of oxygen consumption. However, because of decreased immersion times at higher tidal levels, energy expenditure on aerobic respiration would be reduced for mid tide individuals. Reduced rates of aerial respiration at higher temperatures are thought to minimise water loss and desiccation.



Physiological adaptation to aridity in gerbils (Rodentia: Muridae: Gerbillinae)

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Gerbils are rodents of arid environments. As such water intake is limited and we might expect survival to depend upon physiological and behavioural adaptations to reduce body water flux. In this study we determine several physiological parameters in the bushveld gerbil (*Tatera leucogaster*) of southern Africa and combine and compare these data with previously published data for other gerbil, rodent and mammal species. We conclude that gerbils have, on average, comparatively low basal metabolic rates, average rates of minimum thermal conductance, low rates of resting evaporative water loss at temperatures below thermoneutrality and the ability to produce urine of comparatively high concentration. These findings are discussed in terms of adaptation to environment.

Molecular adaptations in fish hemoglobin function.

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Fishes may experience large temporal and spatial variations in O₂ availability, temperature and other factors that govern blood O₂ transport. These hemoglobins (Hbs) accordingly exhibit greater differentiation in functional properties than those from other vertebrate classes. This includes the presence of Root effects (decreased O₂ binding capacity at low pH that secretes O₂ in the swimbladder and retinal tissues) and the responses to erythrocytic effectors (like ATP) that favour O₂ unloading in the tissues. Moreover, some fish species express electrophoretically cathodic Hb components, which differ from the 'standard' anodic components in having higher affinities, lower Bohr effects and no Root effects, and which may serve to secure O₂ transport under hypoxic and acidotic conditions.

Adaptations in oxygen binding properties of fish blood in response to changes in oxygen tension and temperature are briefly reviewed. The structural traits (amino acid exchanges and intramolecular interactions) that may underly these responses, and the functional differentiation between Hbs from different species, and those between anodic and cathodic components occurring in the same individuals, are discussed.

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Intracellular freezing in *Panagrolaimus davidi*, an Antarctic nematode

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Invertebrates are thought to survive ice formation in their bodies only if it is confined to their body cavities and extracellular spaces. We have demonstrated, however, that *Panagrolaimus davidi*, an Antarctic nematode, will survive intracellular ice formation. We have observed freezing and melting in various intracellular compartments using cryomicroscopy and confirmed the presence of ice using freeze fracture techniques. Individual nematodes which were known to have frozen intracellularly were observed to grow subsequently and reproduce in culture.

Using Differential Scanning Calorimetry (DSC) we have shown that $80.1 \pm 1.9\%$ (mean \pm se) of the total body water of the nematode is converted into ice during freezing. The crystallisation temperature of nematode suspensions was at $-5.8 \pm 0.7^\circ\text{C}$ and melt onset at $-0.9 \pm 0.1^\circ\text{C}$. No post-freeze thermal events were detected during cooling to -40°C and no pre-melt thermal events were detected during warming both at 1°C min^{-1} . The survival of nematodes cooled to -40°C was $89.2 \pm 1.2\%$.

DSC studies on supernatant extracted from nematodes showed no evidence of thermal hysteresis by antifreeze proteins; although recrystallisation inhibition was observed during annealing under a cryomicroscope after splat freezing.

Future studies will focus on the role of ice-active compounds in the survival of intracellular freezing and the location of ice and the disposition of cellular components in frozen nematodes.

Metabolic Depression in Snails, Frogs and Bats: Common Patterns?

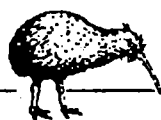
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Metabolic depression to below the normal standard metabolic rate (SMR; ectotherms) or basal metabolic rate (BMR; endotherms) is common amongst a wide variety of animals, in response to a range of environmental stresses including low temperature (hibernation) and high temperature (aestivation).

For example, it is well documented that the metabolic rate of pulmonate snails is dramatically reduced during aestivation to $<10\%$ of the normal SMR; similarly, the metabolic rate of many desert frogs is reduced during aestivation to $<25\%$ of SMR. The absolute metabolic rates of resting snails and frogs are generally similar, as are the metabolic rates of metabolically-depressed animals. Metabolic depression by these ectotherms reflects a basic shift in cellular metabolism to substantially below the normal minimal cellular metabolic rate required by ectotherms. The metabolic depression of aestivating snails and frogs occurs in the absence of any change in body temperature (*i.e.* there is no Q_{10} effect) or substantial hypoxia. Changes in acid-base status ($p\text{CO}_2$, pH) may contribute to metabolic depression, but there is now evidence of intrinsic biochemical metabolic depression as well.

An even more dramatic reduction of metabolic rate has been reported for a variety of mammals and birds during hibernation or aestivation (often to $<1\%$ of the euthermic metabolic rate). However, most or all of the metabolic depression can be explained by their "abandonment" or modification of thermoregulatory control and body temperature *i.e.* by a Q_{10} effect. Changes in thermal conductance may also affect the extent of metabolic depression. The cellular metabolic rate of hibernating bats remains well above even the normal SMR of ectotherms, and is considerably higher than the depressed rate of ectotherms.

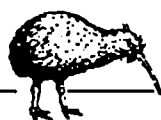


Sodium transport mechanism in postmoult and Na-depleted crayfish *Cherax destructor*

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Na flux in steady state animals acclimated to 0.5 mmol.L^{-1} of Na was $0.4 \mu\text{mol.g}^{-1}.\text{h}^{-1}$ and K_m value was $0.518 \text{ mmol.L}^{-1}$. These values were increased in depleted and postmoult animals by 3.4 and 3.2 times respectively but the K_m value was unchanged. These increases in postmoult and Na-depleted animals were correlated with changes in activity of Na^+K^+ -ATPase and V-ATPase. The activity of Na^+K^+ -ATPase in postmoult animals was 2.6 times that in intermoult crayfish (5.86 and $14.88 \text{ nmol.mg}^{-1}\text{protein.min}^{-1}$ respectively) while activity in Na-depleted animals was 2.2 times the intermoult level at $12.64 \text{ nmol.mg}^{-1}\text{protein.min}^{-1}$. The activity of V-ATPase in the gills of intermoult animals ($3.5 \text{ nmol.mg}^{-1}\text{protein.min}^{-1}$) was also increased in Na-depleted and postmoult animals (8.04 and $9.09 \text{ nmol.mg}^{-1}\text{protein.min}^{-1}$ respectively). This indicates a probable role for this enzyme in Na transport in Na-depleted animals and potentially also in Cl absorption in postmoult animals.



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